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WEIGHT-CARRYING CAPACITY AND EXCITABILITY OF EXCISED CILIATED EPITHELIUM

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The ciliated cell is an example of inherently rhythmical contractile tissue, of a primitive, and therefore fundamentally interesting, type. Nervous modification of ciliary action is said to exist in some species (1), not in others (2). The ciliated pharyngeal epithelium of the frog has been shown to be excitable, both in the intact animal (1) and in the excised membrane (3). The excitability can be elicited by placing particles on the surface (4) or by stroking (3). When unstimulated the cilia are relatively quiescent (4).

Ciliated cells thus possess two qualities; contractility and excitability.

The customary method for measuring the activity of cilia is to place a small particle on the ciliated surface and then to measure the time required for the particle to travel a fixed distance, usually 1 cm. (5, 6). This simple procedure has been used to determine the action of drugs on ciliary action (5). The method is open to the objection that the testing procedure alters the activity of the cilia through excitation.

The purposes of this investigation were *a)* to find the relation between particle area, mass and velocity and *b)* to find the effect of particle mass on the excitability of the ciliated surface.

EXPERIMENTAL METHODS

Preparation of the mucosal surface. A rectangular strip of pharyngeal mucosa was obtained by excision from the superior surface of the frog's pharynx, from the anterior border of the optic bulges to the pharyngo-esophageal junction and laterally to the openings of the Eustachian tubes. The resulting strip was usually about 2 cm. long and 1 cm. wide and was pinned flat, with gentle traction, on the surface of a weighted cork disc. The assembly was immersed in amphibian Ringer's solution.

Velocity measurements. A transparent millimeter scale was pinned a few millimeters above, and parallel to, the membrane surface. The preparation was visualized through a binocular dissecting microscope, the axis of the ciliated membrane being perpendicular to the interocular axis of the microscope; this precaution avoided confusion due to parallax. The passage of a test object was signalled as it passed each millimeter mark, by means of an ordinary signal magnet and kymograph.

Test objects. For the investigation of the relation between area of contact and velocity of the test objects, the objects consisted of discs punched from thin aluminum foil. The discs were of uniform thickness but of different diameters. Thus the load per unit of surface area remained constant, but the actual area varied.

For the investigation of the relation between mass and velocity, the surface of contact was held constant while the load varied. The test objects were aluminum or lead discs 6 mm. in diameter, of various heights necessary to give masses from 0.0005 to 8.0 gm.

TABLE 1. *Relation between surface area and velocity at constant load*

DIAMETER OF DISC	MEAN VELOCITY	STANDARD DEVIATION OF THE MEAN
mm.	*mm./100 per sec.*	
4	75.0	5.8
2	73.3	5.4
4	78.7	7.6
1	70.9	3.5
2	77.1	5.4

Evaluation of results. In calculating results individual measurements of velocity were made at each millimeter for the full centimeter distance. The mean of the 10 velocities in the single run was taken; this gave a more accurate measure of the activity of the membrane than the overall velocity, permitting observation of variations in the activity of different areas of membrane and providing data for the statistical evaluation of differences between runs.

For comparison of individual runs allowance had to be made for the natural deterioration of the preparation. A lead disc of 0.5 gram was taken as a standard test object; every second run was performed with this weight. The velocity indicated the degree of deterioration and variability between duplicate runs. When widely divergent results were obtained in successive standard runs, the intervening test run was discarded.

Results were expressed in two ways, as absolute velocities and also as percentages of the standard runs immediately adjacent.

RESULTS

Relationship between area of contact and velocity at constant load. Using test objects of uniform thickness but different diameters, it was found that the velocity was constant. That is, the force exerted by a unit area of membrane was constant when the load was constant under similar conditions.

Table 1 gives the mean velocities for five successive runs using discs of various diameters. The standard deviations of the mean velocities show that the differences between runs were not significant.

Relationship between weight of test object and velocity at constant area of contact. Using test objects of uniform surface area of contact, but different weights, it was found that the velocity decreased with loads greater than 20 mgm. per sq. mm. The relation between load and velocity was approximately linear. Loads above 140 mgm. per sq. mm. often failed to move entirely.

With loads less than 20 mgm. per sq. mm. the velocities were somewhat more variable. In general the velocity was about the same for all loads between 0.0008 and 20 mgm. per sq. mm., with a slight increase in velocity with increasing loads. In figure 1 the velocity, in percentage of the standard, is plotted against the load, in mgm. per sq. mm.

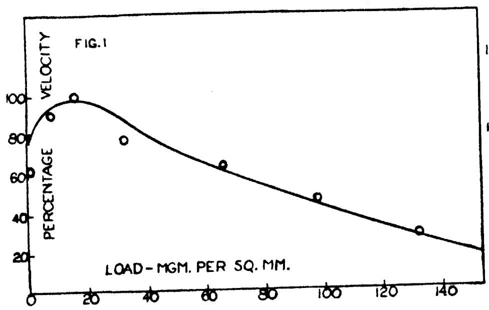

FIG. 1

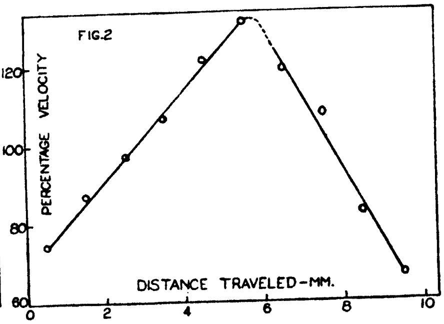

FIG. 2

Variations in velocity at different points on the ciliated surface. As might be expected the velocity was not constant throughout the whole distance travelled by a test object. The object was applied to the membrane about 3–5 mm. anterior to the measuring scale; it accelerated from a standing start and did not reach a steady velocity before measurement began. In general, the velocity continued to increase for the first 6 mm., then decreased for the remaining 4 mm. The low velocity at the end of the membrane was probably due to injury near the cut edge.

In figure 2 the percentage mean velocities at each mm. are plotted against the distance travelled. The velocity at each millimeter was expressed as a percentage of the mean velocity of the run, and these percentages averaged for each millimeter. The plotted points are the mean values for 41 separate runs, using the standard 0.5 gram weight.

The plotted points show an astonishingly close approach to linearity, i. e., the velocity increased at a constant rate for the first 6 mm. In other words, the acceleration was constant, indicating the existence of a constant force acting on the weight.

Relationship between the mass of the test object and the degree of excitation of the membrane. Excitation of the membrane was produced by rubbing the test object back and forth on the membrane in a region anterior to the measured distance used for the velocity measurements. This procedure always resulted in a greatly increased velocity of the test object as compared with the preceding runs in which no excitation was produced. The increases in mean velocities were of the order of 200% over the control runs. This phenomenon was observed with all sizes of test object, even the very heaviest. Thus the excited membrane was able to move objects which were too heavy for the same membrane in an unexcited condition.

Table 2 shows the percentage increase in mean velocity due to excitation for test objects of different weights. After excitation it was observed that the initial velocity was high; that is, the acceleration was greater at the start of the run.

TABLE 2. *The effect of excitation: Increased velocity at different loads*

WEIGHT OF TEST OBJECT	LOAD	INCREASE IN VELOCITY DUE TO EXCITATION
grams	*Mgm. per sq. mm.*	%
0.0003	0.008	230
0.026	0.92	415
0.50	16.4	134
0.50	16.4	142
0.50	16.4	160
1.0	33.0	225
2.0	66.0	268

DISCUSSION

The ciliated surface is evidently capable of moving relatively large loads. Enough energy is available to move any particle of physiological density, even with a small area of contact. The efficiency of ciliary activity must depend on other factors, particularly the state of excitement of the membrane and the degree of traction. The ciliated surface is excited to increased activity by mechanical stimulation; the state of excitement is not confined to the area of stimulation but spreads, particularly in the direction of the ciliary stream.

The ciliated surface may be overloaded by a heavy weight but yet remain in a state of submaximal activity. It follows that the weight of the test object, per se, is not an adequate stimulus for excitement of the activity. On the other hand, movement of even a very light test object to and fro on the surface produces a high degree of excitation.

Direct observations of the ciliary waves by the method of Lucas (1) shows that mechanical stimulation causes an increase in the rate of the beat.

SUMMARY

1. The velocity of test objects, applied to excised ciliated epithelium from the frog's pharynx, is constant for different sizes of particles if the weight per unit area of contact is kept constant.

2. The velocity decreases with increasing load above 20 mgm. per sq. mm.
3. Excised ciliated epithelium is excitable mechanically, but weight alone is not an adequate stimulus.

REFERENCES

(1) Lucas, A.M. This Journal **112**: 468, 1935.
(2) Plattner, F. and C. L. Hou. Arch. f. d. ges. Physiol. **228**: 281, 1931.
(3) Kraft, H. Pfluger's Archiv. **47**: 196, 1890.
(4) Lucas, A. M. Proc. Soc. Exp. Biol. & Med. **30**: 501, 1933.
(5) Boyd, E. M., J. W. Clark and W. F. Perry. Archiv. Otolaryngol. **33**: 909, 1941.
(6) Gray, J. Proc. Roy. Soc. **B95**: 6, 1923.

IMMEDIATE EFFECTS OF ETHER AND NEMBUTAL UPON SOME OF THE BLOOD COMPONENTS IN THE CAT

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The effect of various anesthetics on the volume and composition of the blood has been the subject of numerous investigations in the past. Our attention was drawn to the problem when, in the course of our work, it became imperative to know more accurately what ether and nembutal do to the blood sugar, hematocrit, plasma protein and differential white cell count in the cat and whether or not any of these factors differ significantly in ear and heart blood. No definitive answer could be found in the literature, since these sources were either vague or contradictory concerning some of the values in which we were most interested.

METHODS

In all experiments adult male cats were used. Most of them had been kept under controlled laboratory conditions for many months. Once a day they were fed a varied diet, consisting of meal mixed with water or milk, pellets, fish or meat. Feeding time was usually late in the afternoon after all experimental procedures for the day had been completed.

Prior to anesthesia blood was always drawn from the ear. Before cutting the vein, blood flow was stimulated by rubbing the ear vigorously. The blood dropped freely from the wound into the bottom of a wide-mouthed flask. Heparin was used as an anti-coagulant. The wound was wiped with a wad of cotton whenever clotting appeared imminent. After anesthesia two consecutive blood samples were drawn, from the ear, as before, and from the heart by cardiac puncture. Blood collected from the heart was taken up directly into a dry syringe through a needle inserted in the left ventricle. After withdrawal of the proper amount of blood, the syringe was emptied immediately into a wide-mouthed flask containing heparin.

Drawn blood was centrifuged for 50 minutes in Wintrobe hematocrit tubes to maximal centrifugation. Plasma protein was calculated from the specific gravity, determined by the falling drop method of Barbour and Hamilton (1), as modified by Drew, Scudder and Papps (2). Differential white cell counts were made on smears stained with Wright's stain. The final counts represent the average of two independent counts made by separate individuals. Blood was analyzed for sugar according to the method of Saifer, Valenstein and Hughes (3).

Ether was first administered by placing the cat in an ether box. The reactions of the animals varied. Some were quiet and apparently not too uncomfortable. Others struggled violently. All cats showed considerable salivation. On collapse the animals were removed from the box and ether was administered by cone. Blood was taken immediately thereafter, usually not more than 10 to 15 minutes after anesthesia had begun.

Nembutal was given intra-abdominally, 25 mgm/kgm. The animals lost consciousness without struggle, usually within a few minutes after injection. Blood was withdrawn, as a rule, not more than 10 minutes after injection.

TABLE 1. *The immediate effect of ether and nembutal upon some of the blood components in the cat*

	ETHER			NEMBUTAL		
	Before anesthesia	After anesthesia		Before anesthesia	After anesthesia	
	Ear blood	Ear blood	Heart blood	Ear blood	Ear blood	Heart blood
Hematocrit						
Mean ± σ	30 ± 4.9	39 ±5.4	39 ±4.7	32 ± 8.1	28 ±7.2	28 ±7.5
P		.001	.001		.20	.20
% change		+30	+30		−14	−14
Plasma protein (grams %)						
Mean ± σ	7.02 ± .62	7.47 ±.66	7.44 ±.64	7.11 ± .70	6.67 ±.61	6.66 ±.50
P		.10	.10		.10	.10
% change		+6	+5.6		−6.2	−6.3
White cells/cu.mm. (thousands)						
Mean ± σ	12.9 ± 5.04	17.7 ±9.9	18.2 ±11.4	10.9 ± 4.1	9.5 ±3.7	8.7 ±3.4
P		.1	.1		.35	.15
% change		+37	+40		−13	−20
Lymphocytes (%)						
Mean ± σ	49 ±16.6	51 ±12.9	51 ±14.7	50 ±16.2	49 ±16.2	52 ±13.9
P		.7	.7		.9	.9
PMN (%)						
Mean ± σ	40 ±14.9	39 ±12.5	38 ±15.3	43 ±16.0	44 ±15.9	41 ±12.5
P		.7	.7		.9	.7
Lymphocytes (absolute)/cu.-mm. (thousands)						
Mean ± σ	6.10 ± 3.07	7.68 ±3.94	8.64 ±5.41	6.00 ± 3.19	5.15 ±2.85	4.30 ±1.72
P		.5	.2		.5	.1
% change		+26	+41		−14	−28
PMN (absolute)/cu.mm. (thousands)						
Mean ± σ	5.51 ± 4.36	7.72 ±6.04	7.78 ±5.96	4.20 ± 1.71	3.35 ±1.42	3.76 ±2.11
P		.3	.3		.2	.5
% change		+40	+41		−20	−11
Blood sugar (mgm. %)						
Mean ± σ	68.7 ±24.5	133.4 ±36.5	131.7 ±36.1	79.7 ±14.0	90 ±9.0	96 ±21.8
P		.001	.001		.05	.05
% change		+94	+92		+13	+20

The data were analyzed statistically by the methods described by Davenport and Ekas (4), with the exception of the t values used in deriving the P values (table 1). The following formulae were used in calculating these values:

$$t = \frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}} \qquad \epsilon = \sqrt{\frac{\Sigma(v - m)^2}{n(n - 1)}} \qquad \begin{aligned} m_1 &= \text{mean of } x \\ m_2 &= \text{mean of } y \end{aligned}$$

RESULTS

The data summarized in table 1 show the mean values of some of the blood constituents in unanesthetized cats and the same values in both ear and heart blood after either ether or nembutal anesthesia. The *P* values give the probability that the mean differences observed before and after ether or nembutal anesthesia were due to chance alone. The per cent change refers to the shift in values between the mean of the unanesthetized and anesthetized states.

The ether data were obtained from 14 experiments on 12 different animals and the nembutal data were obtained from 13 experiments on 13 different animals. The individual hematocrit, plasma protein and blood sugar values, with a few unimportant exceptions, change in the same direction as do the average values. In 30% of the observations on changes in polymorphonuclear counts, the individual shifts are opposite to the shift in the mean value; in the lymphocyte counts, 15%. The data on white cell distribution are therefore much less significant than the values for hematocrit, plasma protein and blood sugar determinations. They are included, however, since they are consistent with the general findings.

Ether appears to increase hematocrit, white cell count and plasma protein values, the hematocrit and white cell count proportionately more. The relative number of lymphocyte and polymorphonuclear (PMN) cells remains the same, since the percentage of both remains unchanged in the blood after anesthesia. However, their absolute number, as well as the total white count, increases. This increase is of the same order of magnitude as in the red cells, and suggests that both red and white cells are affected in the same manner. The blood sugar rises sharply, almost doubling its pre-anesthesia level.

Under nembutal the hematocrit, white cell count and plasma protein levels of the blood fall; the hematocrit and white cell count more than the plasma protein value. There is no indication of any relative shift in the proportion of leucocytes, but the absolute number of lymphocytes and polymorphonuclear cells is diminished. The blood sugar values rise under nembutal, but only slightly in comparison with the values under ether.

Samples of ear and heart blood drawn in succession under ether or nembutal appear to be vitually identical in respect to hematocrit, plasma protein and blood sugar values. The same is true under ether in respect to leucocyte and PMN cell count. However, under both ether and nembutal the lymphocyte count shows a greater change in heart blood than it does in ear blood. Under nembutal alone the leucocyte count and the PMN cell count also differ more in heart blood than ear blood, when compared to the counts obtained before anesthesia.

DISCUSSION

In the cat the immediate effect of ether is to produce hemoconcentration. The hematocrit rises about 30% and plasma protein values increase about 6%. The greater rise in hematocrit values can be attributed to the action of the spleen. Numerous investigators, among them Bollman, Svirbley and Mann (5), Barbour (6) and Jarcho (7), have shown that removal of the spleen sharply reduced the increase in hematocrit induced by ether in various species. As shown in table 1, the white cells shift in substantially the same amount and direction as do the red cells. This fact indicates that the spleen contributes proportionately the same number of white and red cells to the blood. The difference in white cell counts has a far lower degree of significance than has the difference in hematocrit values. This observation is an illuminating commentary on the precision of the two methods used to evaluate these factors. The usual counting technics employed in determining the shift in leucocyte counts encountered in this study do not yield results capable of passing the more rigid tests for statistical significance. Hence the hematocrit data have a higher degree of significance than the data for the white cell counts. If this difference is not due to a varying degree of refinement in the methods used, as suggested, then the data must indicate that the white cell count is influenced by factors other than those affecting the hematocrit value.

Nembutal anesthesia leads to hemodilution in the cat, as indicated by the decrease in both red and white cell counts and by a decrease in plasma protein values shortly after induction. Again the greater change in red cell count, as compared to the plasma protein values, can be attributed to the action of the spleen. Jarcho (7) observed that in the splenectomized cat or dog anesthesia produced less shift in red cells than was observed under similar conditions in normal animals, but that plasma protein changes were unaffected.

An attempt to review the literature concerning the effect of anesthesia upon blood volume would be unwarranted at this time. However, a few of the more pertinent observations bearing upon these results may be discussed here. Direct measurements by other workers of blood volumes in the cat and dog confirm, for the most part, the deductions made from our observations. In the dog, ether decreases plasma volume by 6% and amytal increases it by 9%, the hematocrits changing accordingly (5). In splenectomized animals after ether the plasma volumes were decreased even more than in normal animals. McAllister (8) observed that ether reduced the plasma volume in dogs by 11%. Both plasma protein and hematocrit values increased, but the rise in the latter was substantially greater. Hamlin and Gregersen (9) observed an increased plasma volume of about 10% in the cat after nembutal. Conley (10), in disagreement with other workers, reported no change in the plasma volume, hematocrit or plasma protein values in the cat after ether.

In humans Stewart and Rourke (11) have shown that there is an average decrease of 14.9% in the plasma volume after the administration of ether. This change is of the same order of magnitude as that seen in animals. Remarkably, they report no change in plasma protein or hematocrit values. Therefore they assume that some blood must be shunted into a compartment which is unavailable to the dye. Barbour (6) attributed to the spleen the hemoconcentration seen under ether. He also noted an increase in blood volume in the rabbit after amytal and thought the source of the water to be intra-cellular, probably from the liver and central nervous system.

In the rabbit, Blackberg and Hrubetz (12) have reported no change in the blood sugar values after pentobarbital. This finding is contaray to our observations in the cat after nembutal. All authorities agree that ether produces a rise in blood sugar, probably as a result of sympathetic adrenal stimulation.

There is general agreement that inhalation anesthesia increases, and that barbiturates act to decrease, the white cell count. Taylor and Water (13) report a marked and significant rise in the leucocyte count in 85 clinical cases and 5 dogs after administering various inhalation anesthetics including ether. Higgins and Corwin (14) report the leucocyte counts in etherized, splenectomized rats and rabbits to be less than in a normal etherized animal. After amytal, however, the white count was lower in the splenectomized than in the nonsplenectomized rabbit, but in the rat the splenectomized animal showed a higher white count than the nonsplenectomized. Searles (15) reported that in dogs there was an increase in cell volume after ether. This increase was reduced by one half after splenectomy. After amytal, there was a decrease in cell volume in the normal animal, but no change in the splenectomized. Crafts (16) observed no change in the white and red cell counts in the heart blood of the rat after ether, regardless of the depth of anesthesia. He also reported hemoconcentration in the dog, but not in the rabbit or cat, after ether.

SUMMARY

(1) Ether produced an immediate increase in the hematocrit value, plasma protein, blood sugar, leucocytes, lymphocytes and PMN cells of the blood of cats drawn from either the heart or ear. There was no change in the relative proportion of lymphocytes and PMN cells.

(2) Nembutal produced an immediate decrease in the hematocrit value, plasma protein, leucocytes, lymphocytes and PMN cells, and a slight rise in blood sugar in the blood of cats drawn from either the ear or heart. There was no change in the relative proportion of lymphocyte and PMN cells.

(3) Heart blood and ear blood, drawn under either ether or nembutal, showed no significant difference in respect to hematocrit value, plasma protein or blood sugar nor did the proportions of lymphocytes and PMN cells differ. However, under both ether and nembutal, the absolute number of lymphocytes changed more rapidly in heart blood than in ear blood. Under nembutal alone there is also a more rapid change in leucocytes and PMN cells in heart blood than in ear blood.

REFERENCES

(1) Barbour, H. G. and W. F. Hamilton. This Journal **69**: 654, 1924.
(2) Drew, C. R., J. Scudder and J. Papps. Surg., Gyn. and Obs. **70**: 859, 1940.
(3) Saifer, A., F. Valenstein and J. P. Hughes. J. Lab. and Clin. Med. **26**: 1969, 1941.
(4) Davenport, C. B. and M. P. Ekas. Statistical methods in biology, medicine and psychology. Wiley and Sons, New York, 1936.
(5) Bollman, J. L., J. L. Svirbley and F. C. Mann. Surg. **4**: 881, 1938.
(6) Barbour, H. G. Anesthesiol. **1**: 121, 1940.
(7) Jarcho, L. W. This Journal **138**: 458, 1943.
(8) McAllister, F. F. This Journal **124**: 391, 1938.
(9) Hamlin, E. and M. I. Gregersen. This Journal **125**: 713, 1939.
(10) Conley, C. L. This Journal **132**: 796, 1941.
(11) Stewart, J. D. and G. M. Rourke. J. Clin. Invest. **17**: 413, 1938.
(12) Blackberg, S. N. and M. C. Hrubetz. Proc. Soc. Exp. Med. **34**: 63, 1936.
(13) Taylor, I. B. and R. M. Waters. Curr. Res. Anesthesia and Analgesia **14**: 276, 1935.
(14) Higgins, G. M. and W. C. Corwin. Surg. **1**: 703, 1937.
(15) Searles, P. W. J. A. M. A. **113**: 906, 1939.
(16) Crafts, R. C. J. Lab. and Clin. Med. **29**: 1070, 1944.

COMPOSITION OF THE REGENERATING LIVER AFTER PARTIAL HEPATECTOMY IN NORMAL AND PROTEIN-DEPLETED RATS[1]

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The following data were obtained in order to present a more complete picture of the regenerating rat liver than is now available. The older literature on liver regeneration is concerned chiefly with the total liver mass and with morphology and has been reviewed repeatedly (1–3). Relatively little has been published on the composition of the liver after partial hepatectomy, and in no one set of experiments are the data complete for all the main components of the organ.

The pioneer study of Higgins and Anderson (4) on regeneration in terms of total liver mass also included some values for the water component. Brues, Drury and Brues (5), while studying the mitotic rate after partial hepatectomy in rats, determined total nitrogen concentrations. Both these papers suggested some distortion of normal composition during the first two or three days after operation apparently related to disproportionate increases in liver fat. Bogetti (6) and Bogetti and Mazzocco (7) determined the fat, water and nitrogen content of regenerating liver. They found that the liver fat concentration increased sharply for 48 hours after operation, then gradually declined. Similar results were obtained by Ludewig, Minor and Hortenstine (8), who analyzed for neutral fat, phospholipids, cholesterol and cholesterol esters during the first postoperative week. The only lipid fraction which showed a significant change in concentration was the neutral fat, which was laid down most rapidly in the first 48 hours. The behavior of liver lipids after partial hepatectomy has been studied under a variety of circumstances (9–12). The storage of glycogen after partial hepatectomy in the rat has been found to be proceeding in the liver by the third postoperative day (13). Thus some data are available on the water, lipid and glycogen components of the regenerating liver. Concerning the behavior of the liver protein after partial hepatectomy, there is very little known.

Object of experiments. Walter and Addis (14) suggested that the protein content of the liver, rather than the organ's total mass, is the measure of effective liver size. In line with this reasoning we undertook to determine the water, glycogen, lipid and protein content of the regenerating rat liver, with particular emphasis on the protein component. The first series of animals was maintained on a normal diet. In the second series we wished to extend the study to animals in an abnormal dietetic state. Brues, Drury and Brues (5) had reported a considerable degree of regeneration in animals which had been fed glucose alone for 7 days before operation and during the postoperative period up to 4 days. Handler and Bernheim (11) found gross regeneration in the face of continuing body-weight losses on deficient diets. However, there is no information on the composition of the gain under these taxing conditions, nor on the extent to which the liver proteins take part. We elected, therefore, to study the composition of the regenerating liver in a second series of rats previously partially depleted of protein by a period on a protein-free diet. The results derived from these two series, normal and protein-depleted, form the basis of this report.

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MATERIALS AND METHODS

Animals. Male Wistar rats were used, all young adults ranging in initial body weight from 222 to 277 grams. They were divided into groups of 5 or 6 rats with mean weights of approximately 250 grams. The animals were kept in individual cages. All feeding was ad libitum. Daily records of food intake and body weight were kept for each rat once the specific synthetic diet was begun. Maximum and minimum room temperatures were recorded daily and averaged 28°C. with transitory variations from 24 to 31°C. Food and tap water were left in the cages up to the time when the animals were removed for operation or sacrifice. All operative and sacrifice procedures were carried out between 9:30 and 11:30 a.m. Complete notes of necropsy findings were recorded.

Diets. The experimental diets were based upon a non-protein diet of the following composition: sucrose (cane sugar), 91%; cod liver oil (Mead Johnson's Standardized), 3%; celluflour, 2%; salts (McCollum 185), 4%. A vitamin supplement was incorporated to give the following quantities per 10.0 gram unit of food: thiamine, riboflavine and pyridoxine, 0.1 mgm. each; nicotinic acid, 1.0 mgm.; inositol, 6.0 mgm.; paraminobenzoic acid, 2.0 mgm.; calcium pantothenate, 0.6 mgm.; choline chloride, 20.0 mgm. This supplement was blended with 500 grams of sucrose in amounts sufficient for 10.0 kgm. of diet. An appropriate weight of the vitamin mixture was mixed into each lot of diet in substitution for plain sucrose.

A single lot of air-dried casein (453-'Labco') furnished the protein when the basal non-protein diet was converted into a 'normal' diet. This was done by replacing 18.0 grams of sucrose by an equivalent weight of casein. By analysis the casein contained 13.8% nitrogen. All diets were considered to be isocaloric at 3.9 calories per gram. In all mixtures fat contributed only 7% of the calories.

Operation. The operation of Higgins and Anderson (4) was used with minor modifications. Under ether anesthesia a one inch midline incision was made in the upper abdomen. The cartilaginous portion of the xiphoid process was removed, and the left lateral and median lobes, as defined by Higgins and Anderson (4), were delivered. After freeing the ligaments a thread of fine silk was drawn around the common pedicle of these two lobes so as to cut through as little liver tissue as possible. The lobes were then cut free close to the ligature.

Closure was by continuous silk in two layers. No dressing was applied. All operations were handled by the same operator and anesthetist at a fairly constant rate of five per hour. There were two deaths from an overdose of ether and two from bleeding from the stump in the 70 operations reported here.

Indirect estimation of liver size. Brues, Drury and Brues (5) described a method for the indirect estimation of liver size based on the relative constancy of the ratio between the mass of the right lateral plus caudate lobes, which constitute the remnant left in situ at the close of the operation, and the left lateral plus the median lobes, which are removed. They found the 'remnant' to comprise 31.6% ±1.5% of the total liver mass. We adopted their principle of using each animal as its own control but used a slightly different method for obtaining the ratio of remnant to total liver. Twenty-four male rats of the species used in the experiments were anesthetized, and the operation described above was commenced. Within five seconds of tightening the ligature on the lobes to be removed, the large veins between liver and diaphragm were divided. The ligated lobes were at once removed, blotted, sliced and placed in a weighing bottle. The exsanguinated remnant was similarly handled. After weighing, the ratio between the remnant and the whole was calculated.

In terms of total mass the average value for the 24 rats showed the liver remnant to make up 30.6% of the total liver. The highest value was 33.5%, the lowest, 28.1%, the standard deviation, ±1.34%. Following the stipulations of Fisher (15) the results were applied to a frequency distribution curve, and an essentially normal distribution was found. On the basis of this ratio the factor by which the figure for the removed lobes was to be multiplied to give the figure for the total liver was 1.441. This factor was used throughout in computing results, not only for total mass, but for all components.

Analytical methods. At both operation and sacrifice the tissues were handled by the same individual. The removed liver was blotted and freed of excess blood, and after sampling was delivered into appropriate containers for weighing and analysis. The samples for each determination were always taken from the same lobes, approximately 0.3 gram for glycogen, 0.4 to 0.5 gram for solids and 0.3 gram for histological examination. The remainders were pooled for nitrogen determinations and lipid analysis, an attempt being made to use a similar percentage of the total liver tissue from each animal.

Glycogen was determined by the method of Good, Kramer and Somogyi (16). Total solids and water were determined by drying the sample in an oven at 95 to 100°C. for 24 hours.

The dried tissue was then analyzed for total nitrogen by the Kjeldahl method, using a selenium and copper catalyst. The remaining tissue was weighed and dropped in M/2 acetate buffer, pH 5.0, heated to a temperature of 85°C. The pooled tissue was allowed to remain in the hot acetate for 30 minutes. It was then cooled and homogenized in a Waring blender. Aliquots of the homogenate were analyzed for total nitrogen and extractive nitrogen. An aliquot was also taken for the determination of total lipids by a modification of the method of Leathes and Raper (17). The balance of the homogenate was used for protein isolation by the method of Addis (18). When lipid analyses of individual samples were to be made, the tissue was ground with sand, extracted with boiling alcohol-ether, with subsequent weighing of the petroleum-ether soluble fraction.

When the term 'liver protein' is used, it will be meant to signify the acetate insoluble nitrogen multiplied by the conversion factor 6.25. The term is used for convenience only, and it is recognized that it will include nitrogen of other than strictly protein origin, specifically some nucleic acid and phospholipid nitrogen. In the cases where the individual liver protein values are given, the value for the extractive nitrogen obtained from the acetate pool has been subtracted from each total nitrogen reading from the individual samples, the difference being taken as the protein nitrogen.

EXPERIMENTS

Normal (casein-fed) series. Five groups of 5 or 6 rats, 26 animals in all, were used to obtain data on the composition of the liver after partial hepatectomy in normal animals maintained on the 18% casein diet. After operation they were maintained on the same diet until sacrifice. Three animals died at operation. One group was sacrificed at each of the following intervals after operation: 1, 2, 4, 8 and 14 days. Two other groups of 6 animals each were placed on diets containing 27% and 50% of casein, respectively, for 7 days before operation and continued on the same diets postoperatively. Both groups were sacrificed after 14 days. There were no postoperative deaths among the casein-fed animals.

Protein-starved series. Twenty-six rats in five groups were taken off the stock diet and placed on the non-protein diet for 14 days before operation. After operation the same diet was continued. The animals were sacrificed by groups at the same intervals after operation as the casein-fed series. There were no operative deaths, but one rat died 4 days after operation with interstitial pneumonia. A further group of 6 rats was submitted to a period of 28 days of protein depletion before operation. One animal died with evidence of pneumonia on the second day after operation. The remainder were sacrificed 8 days after operation.

RESULTS

All data are summarized in table 1. Comparison of the 18% casein-fed and the non-protein series is facilitated by the diagram shown as figure 1. For the casein-fed groups the body weight shown and used in recalculating all values to a body-weight basis is the weight on the morning of operation. For the protein-starved series the initial body weight is that of the morning on which the depletion diet was started.

The data show that the pre-operative period of 14 days on the non-protein diet resulted in a reduction of average total liver mass from 3.88 grams % of body weight to 2.86 grams %. This reduction is seen to have occurred entirely in the water and protein components, there being no significant reduction in the absolute amounts of glycogen and lipid. The reduction in liver protein as a result of the pre-operative protein starvation amounts of 42%, specifically from

0.70 gram to 0.40 gram per 100 grams of initial body weight. In experiment 7A the preoperative depletion period was extended to 28 days, and liver protein was reduced slightly more to 0.37 gram. These losses of liver protein are similar to those reported after a 7-day fast by Addis, Poo, and Lew (19) and on a protein-

Table 1. *Composition of the regenerating liver in a) 'Normal' (casein-fed) and b) protein-depleted rats*

DIET	EXP. NO.	EXP. PERIOD		NO. OF RATS	INITIAL BODY WEIGHT	TOTAL LIVER MASS[1]		COMPOSITION[1]								NEW LIVER PROTEIN FORMED[1]
								Water		Glycogen		Lipid		Protein		
		Pre-oper.	Post-oper.			Oper.	Sacr.	Oper.	Sacr.	Oper.	Sacr.	Oper.	Sacr.	Oper.	Sacr.	
		days	*days*		*gm.*											
(*a*)	1A	8	1	5	259 ±11	3.96 ±.35	1.68 ±.14	2.77 ±.23	1.19 ±.11	0.22 ±.07	0.05 ±.02	0.12	0.13	0.71	0.28 ±.02	0.06
'Normal'	1B	8	2	5	257 ±15	3.68 ±.38	2.22 ±.16	2.60 ±.26	1.58 ±.13	0.14 ±.06	0.11 ±.01	0.12	0.12	0.70	0.34 ±.03	0.12
18%	1C	7	4	4	256 ±21	4.00 ±.19	2.83 ±.31	2.81 ±.14	2.00 ±.22	0.24 ±.05	0.18 ±.07	0.09	0.11	0.69	0.46 ±.03	0.25
Casein	1D	7	8	4	246 ±6	3.74 ±.25	3.42 ±.23	2.61 ±.16	2.39 ±.17	0.20 ±.04	0.25 ±.04	0.12	0.10	0.69	0.58 ±.03	0.37
	6	7	14	5	275 ±14	4.02 ±.54	3.21 ±.22	2.79 ±.30	2.23 ±.15	0.28 ±.12	0.13 ±.04	0.12 ±.02	0.12 ±.02	0.71 ±.06	0.59 ±.05	0.37 ±.03
Operation average				23	259 ±16	3.88 ±.36		2.72 ±.25		0.22 ±.08		0.[illegible]1		0.70		
27% Casein	9	7	14	3	261 ±14	3.44 ±.29	2.97 ±.23	2.38 ±.20	2.03 ±.15	0.16	0.17	0.13	0.08	0.66 ±.03	0.57 ±.01	0.37 ±.02
50% Casein	10	7	14	6	261 ±18	3.42 ±.45	3.31 ±.27	2.38 ±.30	2.29 ±.20	0.13	0.15	0.12	0.11	0.68 ±.08	0.65 ±.06	0.44 ±.04
(*b*)	3A	14	1	5	245 ±17	2.76 ±.31	1.20 ±.08	1.95 ±.21	0.86 ±.08	0.31 ±.04	0.05 ±.02	0.10 ±.01	0.09 ±.01	0.39 ±.02	0.16 ±.01	0.04 ±.00
'Depletion'	2A	14	2	5	242 ±15	2.95 ±.34	1.58 ±.18	2.09 ±.27	1.12 ±.13	0.24 ±.07	0.10 ±.07	0.14	0.10	0.41 ±.03	0.22 ±.01	0.10 ±.01
Non-protein	3B	14	4	5	247 ±11	2.68 ±.25	1.81 ±.17	1.88 ±.16	1.30 ±.11	0.29 ±.11	0.12 ±.06	0.11 ±.03	0.10 ±.03	0.38 ±.03	0.26 ±.02	0.14 ±.01
	2B	14	8	5	243 ±20	3.17 ±.37	2.39 ±.22	2.28 ±.29	1.71 ±.15	0.25 ±.06	0.23 ±.07	0.11	0.08	0.43 ±.04	0.29 ±.02	0.16 ±.01
	7	14	14	5	254 ±11	2.75 ±.17	2.17 ±.11	1.94 ±.14	1.54 ±.07	0.18 ±.05	0.14 ±.02	0.14 ±.02	0.14 ±.02	0.41 ±.01	0.29 ±.02	0.17 ±.01
Operation average				25	246 ±17	2.86 ±.35		2.03 ±.25		0.25 ±.08		0.12		0.40 ±.03		
	7A	28	8	5	259 ±12	2.69 ±.38	2.01 ±.17	1.89 ±.26	1.42 ±.15	0.16 ±.05	0.12 ±.03	0.23 ±.05	0.15 ±.03	0.37 ±.03	0.27 ±.02	0.15 ±.02

[1] All liver analyses expressed in grams per 100 grams initial body weight. Values shown are averages for each experimental group. Where individual values were determined, the standard deviation is shown. S.D. = $\sqrt{\frac{\Sigma\Delta x^2}{n-1}}$.

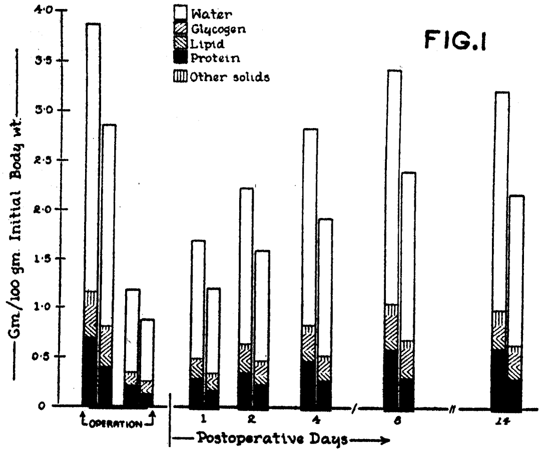

FIG. I

Restoration of Liver Protein after Partial Hepatectomy

FIG. 2

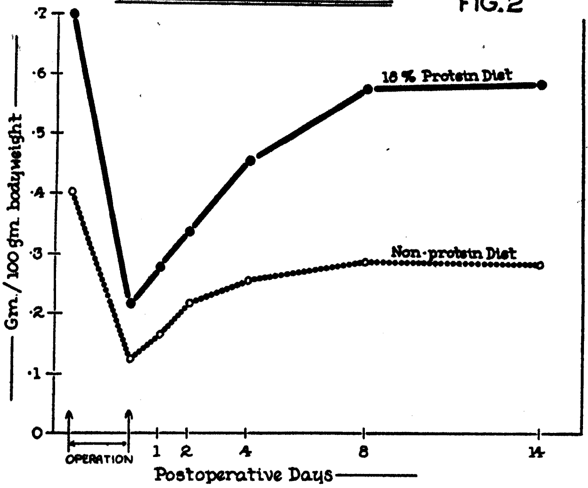

FIG. 1. COMPARISON OF AMOUNT AND COMPOSITION OF THE REGENERATING LIVER IN CASEIN-FED AND PROTEIN-DEPLETED RATS. The taller of each pair of columns is the casein-fed group; the shorter is the protein-depleted. On the left the values at the beginning and end of the operation are shown.

FIG. 2. COMPARISON OF LIVER PROTEIN VALUES FOR THE CASEIN-FED AND PROTEIN-DEPLETED SERIES. Values at beginning and end of operation are shown on the left. Note that the animals on the non-protein diet came to operation with the liver protein reduced from 0.70 to 0.40 gram per 100 grams initial body weight as a result of the pre-operative period of 14 days on the non-protein diet.

free diet by Kosterlitz and Campbell (20). It is readily calculated that the superimposition of 70% hepatectomy upon such protein-depleted livers, although reducing the total liver cells by only 70%, resulted in a net reduction of total liver protein of 83%, as compared with the livers of the normally nourished animals.

To turn to the composition of the regenerating liver as shown by the values obtained at sacrifice, one sees that as regeneration progresses the trend was for a general rise in the absolute amounts of all components to occur. This trend was most consistent in the protein component and was emphasized by the inclusion of a column at the right of table 1, which shows the increment of new liver protein formed for each group. Such increments were calculated by subtracting the value for the remnant at the close of operation (30.6% of the calculated value for the total liver) from the value obtained at sacrifice. For the 18% casein-fed series the liver protein was 0.22 gram at the close of operation and 0.59 gram after 14 days, an increment of 0.37 gram. For the protein-starved series the figure was 0.12 gram at the close of operation and 0.29 after 14 days, giving a gain of 0.17 gram. The difference in the magnitude of the increment in the two series is attributable to the dietary protein. In figure 2 the absolute values for liver protein are compared graphically.

Mention should be made of the movements of the glycogen and lipid components during regeneration. In both series glycogen fell in the first postoperative day. This fall was probably related in part to a voluntary reduction in food consumption which usually occurred at this stage. From the second day on the deposition of glycogen is seen to be proceeding unimpaired. The greater part of the lipid increment was laid down in the first 24 hours after operation. Little more was added on later days although the non-protein series showed a secondary rise at the 14th day.

In both series there was little or no increase in liver protein between 8 and 14 days. In order to see whether more complete regeneration of this component would be achieved by raising the protein intake, experiments 9 and 10 (table 1) were undertaken. The dietary casein levels were raised to 27 and 50% respectively, and the animals were sacrificed after 14 postoperative days. The 27% casein diet produced no significant increase in liver protein increment. The increment achieved with the 50% casein diet was 0.44 gram and resulted in the most nearly complete regeneration of liver protein attained by any group.

In experiment 7A the pre-operative period on the non-protein diet was extended to 28 days. Despite this greater degree of pre-operative depletion, the liver protein increment after 8 days of regeneration was only slightly lower than in the corresponding group depleted for only 14 days before operation.

Table 2 presents the average values for the gross chemical composition of the liver for both 18% casein-fed and protein-depleted series. In this table the values are calculated in grams percent of wet liver weight. At no stage of regeneration was there any striking departure from the percentage composition of the control values at the time of operation. The liver protein concentration in the protein-depleted series was consistently depressed while glycogen and lipid were moderately elevated. It is apparent that no indication of the magnitude of regeneration can be obtained from such percentage figures.

Histological sections were made from samples of liver obtained at operation and sacrifice. These were fixed in formalin and stained with haematoxylin and eosin and with Sudan III. The operative samples revealed nothing abnormal, except for loss of heavy staining properties and increased vacuolization of the cytoplasm in the protein-depleted animals. At sacrifice there was no evidence of necrosis or cirrhosis in the animals of either series. Histological fat was somewhat increased at one and two days after operation, but the increase was never of the extreme order which has been described by other workers. Mitotic activity was most intense after one and two days in both series. After four days mitoses were less numerous, and at later intervals became difficult to find. These observations were in accord with those of Brues and his associates (5), with the exception that in our animals mitotic activity was at least as great at 24 hours as at 48 hours. This led to the question as to whether the latent period after operation before the onset of mitosis might not be considerably less than 24 hours in our animals. In order to test this question 6 casein-fed and 6 protein-depleted rats were operated upon, and 2 from each group were sacrificed at 6, 12 and 18 hours after operation. No animal showed mitoses although the nuclear changes described by Brues *et al.* as being premitotic were evident at 18 hours in both groups. It was concluded that active mitosis must begin rather abruptly between 18 and 24 hours after operation in both normal and protein-depleted animals.

TABLE 2. *Concentrations of components during liver regeneration of normal (casein-fed) and protein-depleted rats*

(Grams per 100 grams of liver)

	CONTROLS		POSTOPERATIVE DAYS									
			1		2		4		8		14	
	Norm. (23)[1]	Depl. (25)	Norm. (5)	Depl. (5)	Norm. (5)	Depl. (5)	Norm. (4)	Depl. (5)	Norm. (4)	Depl. (5)	Norm. (5)	Depl. (5)
Water	70.1	71.0	70.5	71.6	71.2	71.0	70.7	71.9	70.0	71.8	69.5	71.0
Glycogen	5.7	8.7	2.7	4.0	5.2	6.5	6.4	6.4	7.3	9.7	4.1	6.6
Lipid	3.0	3.9	7.4	7.2	5.5	6.1	4.0	5.4	3.1	3.4	3.9	6.3
Protein	18.0	14.0	16.5	13.6	15.2	13.8	16.1	14.1	16.8	12.0	18.3	13.2
Total	96.8	97.6	97.1	96.4	97.1	97.4	97.2	97.8	97.2	96.9	95.8	97.1

[1] Figures in brackets show number of rats averaged.

DISCUSSION

The data presented on the composition of the liver during regeneration constitute the first simultaneous analyses for water, glycogen, lipid and protein. The distortion of normal composition suggested by the data of Higgins and Anderson (4) and of Brues, Drury and Brues (5) was not encountered in our animals. The acute and severe fatty infiltration usually observed after partial hepatectomy was limited to a maximum average total liver lipid of 7.4%. We interpret these lipid values as being related to the low fat and high choline content of our diets.

It will be noted that we have chosen to report results in grams in preference to percentages of liver weight. In experimental work on the liver, especially in the rat where the liver mass can be readily determined, a plea is made for reporting all analytical values in absolute amounts. The advantages of reporting values from the blood in terms of total circulating mass are now well recognized. The same applies to a tissue so labile in its mass and composition as the liver where percentage concentrations may be extremely difficult to interpret and may at times give a false impression of the direction of changes, let alone their order of magnitude.

The series fed casein at the 18% level had regained their pre-operative body weight by the 6th or 7th day. At 14 days the rats averaged 5.8% above their weight at operation. Despite this gain the liver protein regeneration was only 82% complete. The feeding of 50% casein led to a terminal body weight at 14 days which was 7.3% above the body weight at operation with the liver protein 96% replaced. These findings may support the suggestion of Harrison and Long (21) that complete regeneration of liver protein must await the return of the animal as a whole to the pre-experimental level of protein nutrition.

The outstanding fact demonstrated by the protein-starved series was the proof of regeneration of liver protein independent of dietary protein. The liver protein after 14 days of regeneration had increased two and one quarter times from the content of the remnant at the close of operation and stood at 78% of the level found for unoperated controls after 28 days on the non-protein diet. The fact that the liver protein reached a plateau (figure 2) after 8 days suggests that an equilibrium was reached between the liver and the body tissues when a certain level of regeneration was attained. One may postulate that the demands of other body tissues for minimal subsistence quantities of the available endogenous protein ultimately counterbalanced the emergency needs of the liver to replace its own deficiency. This may constitute another example of the principle of dynamic equilibrium (22). Our data also contain some evidence that the rate of regeneration may be greater in the case of greater need. The most rapid rate of regeneration was during the first two postoperative days in both series. However, in terms of percentage increase the rate of appearance of new liver protein was greater in the protein-starved than in the casein-fed series. This suggests a more potent stimulus to liver protein recovery in the animals with a more severe reduction of liver substance.

The partially-depleted, partially-hepatectomized rat is considered to present considerable advantages for the study of internal re-allocation of nitrogenous material from the body to a specific tissue. The dissipation of the more labile tissue protein stores served to standardize the preparation, and one is able to deal with internal movements of nitrogen at a stage when no tissue can be expected to possess more than it can readily spare. The operation of 70% removal of the attenuated liver produces a more severe reduction than when this convenient operation is imposed upon a liver in the protein-fed state. Rats so prepared by one of us have been used by Doctor Drabkin (23) to demonstrate that the regeneration of cytochrome-C may occur on a non-protein postoperative diet. We have used the preparation to study the effect of dietary protein introduced after operation. These results will be reported separately.

SUMMARY

1. The gross chemical composition of the liver during regeneration after 69.4% hepatectomy has been studied at postoperative intervals of 1, 2, 4, 8 and 14 days. Two series of rats are reported. The first or 'normal' series was fed a diet high in carbohydrate, low in fat, and containing 18% casein throughout the experiment. The second or 'protein-depleted' series was fed a similar but non-protein diet for 14 days before operation and after operation to the day of sacrifice.

2. It was found that during the 14-day interval both series regenerated liver tissue which was essentially normal in gross composition. Water, glycogen, lipid and protein were all incorporated into the new liver substance in relatively normal proportions, though at varying rates. Lipid was found to be incorporated almost entirely in the first day. Glycogen deposition was resumed from the second day on.

3. The rate of appearance of new liver protein was most rapid during the first two days in both series, but more rapid in the protein-starved than in the casein-fed animals. After two days the rate slowed markedly in the protein-starved animals and became slower than in the casein-fed.

4. The 18% casein-fed animals formed 0.37 gram of new liver protein per 100 grams of initial body weight in 14 days; the protein-starved animals formed 0.17 gram. The difference is considered to be due to the dietary protein consumed by the former group. Nevertheless, the increment formed by the protein-starved animals represented an increase of almost two and one-half fold over the liver protein present at the close of the operation, and must have been re-allocated from other sources of internal body nitrogen.

5. A casein intake of 50%, both before and after operation, resulted in the largest increment of new liver protein in 14 days, namely 0.44 gram. The increase was sufficient to raise the liver protein to within 5% of the initial level at operation.

6. A pre-operative depletion period up to 28 days was found to be compatible with survival after 70% hepatectomy. Liver protein regeneration after 8 days of a continuation of the non-protein diet was not significantly depressed below levels observed with a 14-day pre-operative depletion period.

7. Histological evidence of mitotic activity appeared between 18 and 24 hours after operation in both 'normal' and protein-depleted animals and was still active after 48 hours but had begun to subside by the fourth day.

8. Attention is drawn to the ability of the partially protein-depleted, 70% hepatectomized rat to regenerate liver protein in the face of continued protein starvation. Such a preparation may have potential value in the study of tissue protein synthesis by experiments in which it is desired to exclude both the more 'labile' protein stores and the influence of dietary protein.

REFERENCES

(1) Fishback, F. C. Arch. Path. **7**: 955, 1929.
(2) Sulkin, N. M. Am. J. Anat. **73**: 107, 1943.

(3) Mann, F. C. J. Mt. Sinai Hosp. **11**: 65, 1944.
(4) Higgins, G. M. and R. M. Anderson. Arch. Path. **12**: 186, 1931.
(5) Brues, A. M., D. R. Drury and M. C. Brues. Arch. Path. **22**: 658, 1936.
(6) Bogetti, H. Rev. Soc. argent. de biol. **15**: 274, 1939.
(7) Bogetti, H. and P. Mazzocco. Rev. Soc. argent de biol. **15**: 285, 1939.
(8) Ludewig, S., G. R. Minor and J. C. Hortenstine. Proc. Soc. Exper. Biol. & Med. **42**: 158, 1939.
(9) Selye, H., J. B. Collip and D. L. Thompson. Lancet **2**: 297, 1935.
(10) MacKay, E. M. and H. O. Carne. Proc. Soc. Exper. Biol. & Med. **38**: 131, 1928.
(11) Handler, P. and F. Bernheim. J. Biol. Chem. **148**: 649, 1943.
(12) Flock, E. V. and J. L. Bollman. Fed. Proc. **5**: 133, No. 1 (Part II), 1946.
(13) Stone, C. S., Jr. Arch. Surg. **31**: 662, 1935.
(14) Walter, F. and T. Addis. J. Exper. Med. **69**: 467, 1939.
(15) Fisher, R. A. Oliver and Boyd, London, 1932.
(16) Good, C. A., H. Kramer, and M. Somogyi. J. Biol. Chem. **100**: 485, 1933.
(17) Leathes, J. B. and H. S. Raper. Longmans, Green and Company, London, 1925.
(18) Addis, T., L. J. Poo, W. Lew and D. Yuen. J. Biol. Chem. **113**: 497, 1936.
(19) Addis, T., L. J. Poo and W. Lew. J. Biol. Chem. **115**: 111, 1936.
(20) Kosterlitz, H. W. and R. M. Campbell. J. Physiol. **104**: 16*P*, 1945.
(21) Harrison, H. C. and C. N. H. Long. J. Biol. Chem. **161**: 545, 1945.
(22) Madden, S. C. and G. H. Whipple. Physiol. Rev. **20**: 194, 1940.
(23) Drabkin, D. L. Fed. Proc. **6**: 249, No. 1 (Part II), 1947.

PROTECTION AGAINST ACCELERATORY FORCES BY CARBON DIOXIDE INHALATION[1]

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Kleinschmidt (1) and Ruff (2) have indicated that carbon dioxide added to respiratory gases increased the tolerance of animals to acceleration. No experimental results are reported, but Ruff (3) stated that 1½–2 g increase in tolerance to acceleration was obtained by means of carbon dioxide administration. The latter investigator believed the effect to be due to vasoconstriction. A publication by Ruff and Strughold (4) quote the above work, and these authors add that the beneficial gas mixtures contained 6 to 7% carbon dioxide. Britton *et al* (5) demonstrated and increased survival of rats which had inhaled carbon dioxide before centrifugation.

The purpose of this work was to make a quantitative physiological investigation of positive g forces. A preliminary report to these experiments has appeared earlier (14).

METHODS

Monkeys, cats and dogs were used as experimental animals. In all about 250 tests were made. Exposures were made on the centrifuge described by Britton *et al.* (6). Maximum acceleration was reached within 3 seconds and deceleration required 2 seconds. Blood pressures and electrocardiographs were continuously recorded by methods previously reported (6). Brachial arterial pressures were measured with the arm 90° abducted; all pressures reported are mean arterial readings. The experimental acceleration varies between 1–7 positive g applied for 5 to 30 seconds.

The respiratory gas mixtures were prepared by adding carbon dioxide to oxygen in a pressure storage tank on the centrifuge. These mixtures contained between 5 and 20% carbon dioxide and they were administered by means of a mask taped over the face of the animal. Control determinations were made by forcing air through the mask and also with mask removed; there was no difference in the results obtained by these two procedures. Pure oxygen was administered in several cases and resulted in no substantial difference from the controls breathing ordinary air. It was therefore believed that the subjective effects of the experimental conditions were negligible, and that high oxygen concentrations per se offered no circulatory protection to the animals in these experiments.

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Virginia.

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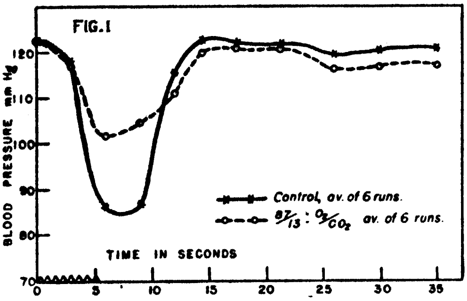
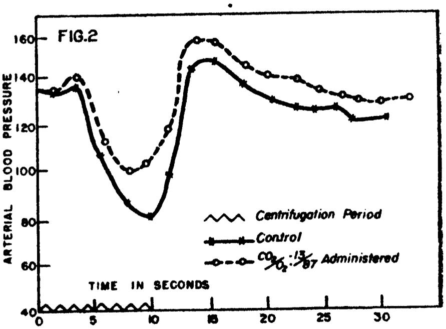
EXPERIMENTAL

Concentrations of carbon dioxide in the order of 5 to 10 volumes % did not appear to be very effective in preventing the blood pressure drop which occurred during 4–6 g exposure. This report includes, therefore, only those results





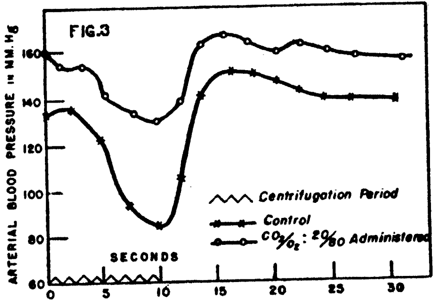


FIG. 1. TYPICAL BRACHIAL ARTERIAL PRESSURE OF A DOG DURING AND AFTER 6 +G FOR 5 SECONDS. Solid line, animal breathing air; broken line, CO_2:O_2 mixture administered for 45 seconds before and 5 seconds during the acceleration. Centrifugation period, 0–5 seconds.

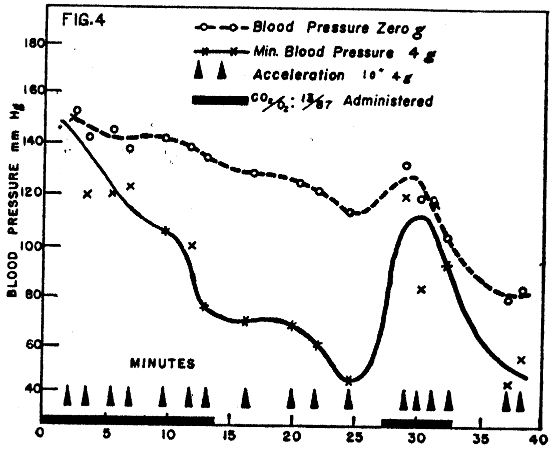
FIG. 2. BLOOD PRESSURE CHANGES IN BRACHIAL ARTERY OF TYPICAL MONKEY CENTRIFUGED AT 6 +G FOR 10 SECONDS. Solid line, average of 6 runs. Broken line, average of 5 runs with CO_2:O_2 mixture administered 15 seconds before and 10 seconds during the acceleration.

FIG. 3. BRACHIAL ARTERIAL PRESSURE OF MONKEY EXPOSED TO 6 +G FOR 10 SECONDS. Open circles indicate effect during inhalation of 20% CO_2 for 25 seconds before and the 10 seconds during centrifugation. Curves represent averages of 6 runs with animal breathing air and 6 runs while breathing the CO_2 mixture.

obtained with administration of 13–20% carbon dioxide; this concentration proved most effective when given 10 to 120 seconds before the acceleration and during the g-subjection period. The animals were usually allowed to breathe air after the acceleratory exposure.

The minimal blood pressure during acceleration was higher when 13% carbon dioxide was administered than when atmospheric air was inhaled. Figure 1 demonstrates that dogs did not exhibit any change in basal blood pressure when a 13% CO_2:87 % O_2 mixture was inhaled. Yet the minimal blood pressure during acceleration was 18 mm. greater than during the control exposures. In these cases the carbon dioxide mixture was administered 45 seconds before and the 5 seconds during acceleration.

Some of the animals did show changes in basal blood pressure during or after the carbon dioxide ventilation. Figure 2 shows data derived from a monkey in which the basal blood pressure was increased at the end of the experiment. The carbon dioxide mixture was administered 15 seconds before and 10 seconds during the centrifugation. Air was readmitted at the end of the acceleration, but basal pressure continued to rise to a level 10 mm. above the control after cessation of carbon dioxide administration. The minimum blood pressure during acceleration with carbon dioxide was 22 mm. above the minimum obtained during control centrifugation.



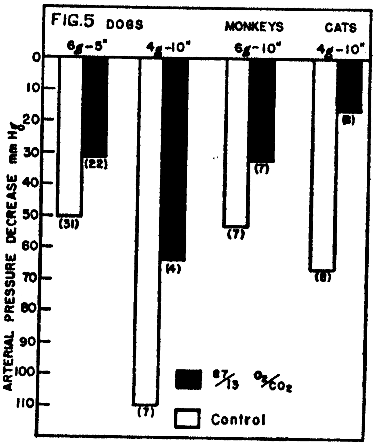


FIG. 4. PROLONGED INHALATION OF 13% CARBON DIOXIDE WITH REPEATED EXPOSURES TO 4 G FOR 10-SECOND PERIODS. The arterial pressure immediately before each centrifugation is shown by a circle (○). The minimum arterial pressure during acceleration is represented by the cross (x). Protective effect of carbon dioxide appears to be reduced after prolonged inhalation of this gas mixture.

FIG. 5. SUMMARY OF BRACHIAL ARTERIAL PRESSURE CHANGES DURING ACCELERATION. Figures in parentheses indicate number of cases.

Higher concentrations of carbon dioxide had greater effects on basal blood pressure, as shown by figure 3. Twenty % CO_2:80% O_2 was administered 25 seconds before and 10 seconds during the centrifugation. In this case the basal arterial pressure was raised about 20 mm. by the carbon dioxide, and the minimum was 45 mm. above that of the control run.

The minimal brachial arterial pressure during acceleration was greater with carbon dioxide than without this gas, whether or not the basal pressure was affected. The 'protection' therefore may be due to more factors than simple vasoconstriction.

Figure 4 indicates that 'protection' of cats by inhalation of carbon dioxide-rich mixtures may be less if this gas is continued for more than 7–12 minutes. Under such conditions the high concentration of carbon dioxide may be harmful, as shown by the continued fall in basal blood pressure of this animal. This figure indicates the typical results demonstrated by one of three cats exposed in this manner.

Optimal 'protection' for monkeys was obtained after inhalation of 13% CO_2:87 % O_2 for 30–60 seconds before a 10-second acceleration exposure, and the gas continued throughout the centrifugation. Dogs and cats required slightly longer inhalation periods. One to two minutes of carbon dioxide administration was often necessary to reduce the g effect on blood pressure in these animals.

Figure 5 summarizes the results obtained from dogs, cats and monkeys under optimal exposure conditions. In some of these experiments basal blood pressure was increased by carbon dioxide, while in others it was not affected. The minimum blood pressure during acceleration was always higher in the case of the carbon dioxide-treated animals than in the case of the controls.

DISCUSSION

The effect of positive acceleration in reducing the brachial arterial pressure of experimental animals is strikingly inhibited by inhalation of carbon dioxide-oxygen mixtures. Thirty to 60 seconds usually elapsed before 'protective' effect of the gas could be demonstrated. It was found most effective to continue administration of the gas mixture throughout the acceleratory period.

It may be that the effects demonstrated were due to specific circulatory shifts rather than a general change in basal blood pressure. Some of the physiological changes which have been reported to be due to high carbon dioxide concentrations are: constriction of splanchnic vessels (7), peripheral vasodilation by direct action of CO_2(8), greater cephalic blood flow (9–11) and increased activity of the carotid sinus (12). Thus the blood may be partially prevented from 'pooling' in the splanchnic area and more blood may be contained in the peripheral capillaries. Since the 10% carbon dioxide mixture results in a greatly increased cephalic blood flow, there may occur a more rapid recovery of cerebral function after positive acceleration in the presence of carbon dioxide.

Dripps and Comroe (13) have recently reported some of the effects produced in human subjects by administration of high concentrations of carbon dioxide. It is believed that the work reported here justifies further investigation of this effect on human beings exposed to acceleratory forces. This method may be combined with other anti-gravity procedures to aid aviators and other personnel exposed to high acceleratory forces, especially when an increased tolerance of 1–2 g may be critical. At high altitude other factors may have to be considered; for instance, at low barometric pressures the percentage concentration of carbon dioxide may have to be increased to maintain adequate partial pressure of this gas.

SUMMARY

The blood pressure changes produced by positive acceleration up to 6 g for 10 seconds were much less severe when animals (monkeys, cats and dogs) inhaled

13% CO_2:87 % O_2 before and during centrifugation. Carbon dioxide administration resulted in about the same protection as that provided by an abdominal belt inflated to 300 mm. of mercury pressure. If administration of 13% carbon dioxide is prolonged up to 7 minutes, the protective effect may be reduced or lost.

We wish to acknowledge the invaluable advice and cooperation of Doctors S. W. Britton and V. A. Pertzoff and the technical assistance of C. R. French.

REFERENCES

(1) KLEINSCHMIDT, A. Luftfahrtmed. **1**: 203, 1936.
(2) RUFF, S. v. Wein. klin. Wochschr. **52**: 861, 1939.
(3) RUFF, S. v. Med. Klin. **36**: 69, 1940.
(4) RUFF, S. v. AND H. STRUGHOLD. Grundriss der Luftfahrtmed., Barth, Leipzig, 1939.
(5) BRITTON, S. W., E. L. COREY AND G. S. STEWART. This Journal **146**: 33, 1946.
(6) BRITTON, S. W., V. A. PERTZOFF, C. R. FRENCH AND R. F. KLINE. This Journal **150**: 7, 1947.
(7) MATHISON, G. C. J. Physiol. **42**: 293, 1911.
(8) PINKSTON, J. O., P. F. PARTINGTON AND A. ROSENBLUETH. This Journal **115**: 711, 1936.
(9) COBB, S. AND F. FREMONT-SMITH. Arch. Neurol. and Psychiat. **26**: 731, 1931.
(10) LENNOX, W. G. AND E. L. GIBBS. J. Clin. Invest. **11**: 1155, 1932.
(11) SCHMIDT, C. F. AND J. C. PIERSON. This Journal **108**: 241, 1934.
(12) GELLHORN, E. Autonomic regulation, Interscience Publishers, Inc., New York, N. Y. p. 25, 1943.
(13) DRIPPS, R. D. AND J. H. COMROE. This Journal **149**: 43, 1947.
(14) VAN MIDDLESWORTH, L. AND S. W. BRITTON. Fed. Proc. **5**: 107, 1946.

CLEARANCES AS A MEASURE OF RENAL FUNCTION IN THE RAT

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The measurement of renal function is most precisely accomplished by recently developed methods for measuring the rate of glomerular filtration, the rate of blood flow through the kidney, and the effective tubular mass. However, these measurements are complicated by their dependence upon both structural and functional factors. Thus glomerular filtration may be expected to vary with the number of glomeruli and the total surface area of the glomerular membranes. But it will also vary with the filtration pressure and the rate at which blood flows through the glomerular capillaries. The maximum excretory capacity will likewise vary with the number and mass of tubule cells, as well as the rate at which blood flows through the post-glomerular capillaries and the presence of chemical components of transport systems. In order to compare renal function or structure from individual to individual, or in the same individual at different times, it is necessary to obtain constant conditions with respect to both the structural and functional factors that affect such measurements.

The variability in functional factors induced by the disturbance to which a rat must be subjected, by virtue of its size in order to obtain clearance measurements, probably accounts for the wide variation in the average values reported by different investigators who performed their experiments under widely differing conditions. It is reasonable to believe that the most satisfactory conditions are those that give the highest clearance values, and hence approximate maximal renal function.

The present investigation was undertaken in order to determine the effect of different conditions on the magnitude of various renal clearances.

METHODS

In this investigation 667 albino rats of both sexes were used.

Creatinine was measured by the method of Folin and Wu, with the slight modifications described by Barrett and Addis (1). Urea was determined by a urease titration method (2). P-aminohippurate (PAH) determinations were performed by the method of Smith and others (3). Blood volume determinations were performed as described by Lippman (4).

Two different sets of conditions were examined. The 'undisturbed' clearance was performed in this way: a group of 10 to 12 rats, uniform with respect to size and sex, were taken off stock diet at 4:00 P.M. on the day preceding the experiment and given 10 per cent dextrose in 0.4 per cent sodium chloride solution with 1 per cent of a vitamin B complex added. In the morning at zero hour, each rat was injected subcutaneously with 10 cc. of 0.85 per cent sodium chloride solution containing 50 mgm. creatinine, 300 mgm. urea, 75 mgm. sodium PAH, or a combination of these. The injection was performed under light ether anesthesia. At the end of a given period, the rat was obliged to sniff ether and empty its bladder, after which urine collection was started. One hour later the urine collection was ended in the same manner. The rat was immediately exsanguinated by severing the abdominal aorta under ether anesthesia. The kidneys were removed and weighed. For the creatinine and urea clearances, collection was timed so that the rats were killed from 1½ to 4½ hours after injection. PAH is cleared from the blood so rapidly that it was necessary to end the collection and kill the rats 1½ hours after injection. Collection of urine was made in individual cages with one-fourth-inch wire mesh floor, placed over a funnel. The funnel was placed over a glass egg, so that urine flowed around the egg into a centrifuge tube supported in an Erlenmeyer flask, but feces were deflected. The glassware was modified by specially fashioned protuberances so that the urine would not overflow at the points of contact. After collection, the egg was washed and the washings joined in the urine pool.

From the above procedure, a rate of excretion and an end serum concentration were obtained. By following this procedure and killing for blood samples at 30-minute intervals after injection, the rate of fall in serum concentration was determined for each of the substances used. In the case of urea and PAH, the log concentration was found to vary inversely with time. In the case of creatinine, this relation did not obtain, but over an hour period the line could be considered straight for practical purposes (fig. 1).

Using the end serum concentration, and assuming a rate of fall that was logarithmic in relation to time, it was possible to calculate a midpoint serum concentration for use in deriving the clearance.

The other set of conditions was designated the 'tailcutting' clearance. The method has been described in detail (5) and differs from the 'undisturbed' clearance in that a sample of blood is obtained by tailcutting prior to the collection period. In this way we know the serum concentration at the beginning and end of the clearance period, so that the midpoint concentration may be calculated, assuming a logarithmic rate of fall.

Use of groups for such determinations has been discussed previously (5). This permits more accurate chemical determinations while keeping shock at a minimum, since only a small volume of blood is required from each individual.

We have expressed clearances as per unit kidney weight. In this way, as we are dealing with normal rats, the structural factors are best reduced to constancy. We also present the clearances in terms of body weight for purposes of comparison with the results of other observers.

RESULTS

The mean results are summarized in table 1. Details concerning each group appear in tables 2, 3 and 4.

It appears clear that the conditions of 'tailcutting' reduce the creatinine and urea clearances to about two thirds of the values found under 'undisturbed' conditions. The rate of plasma flow through the kidney, as indicated by the PAH clearance, also falls, though to a lesser degree.

Within the group of determinations under each set of conditions, there did not appear to be any relation between urine flow and clearance value.

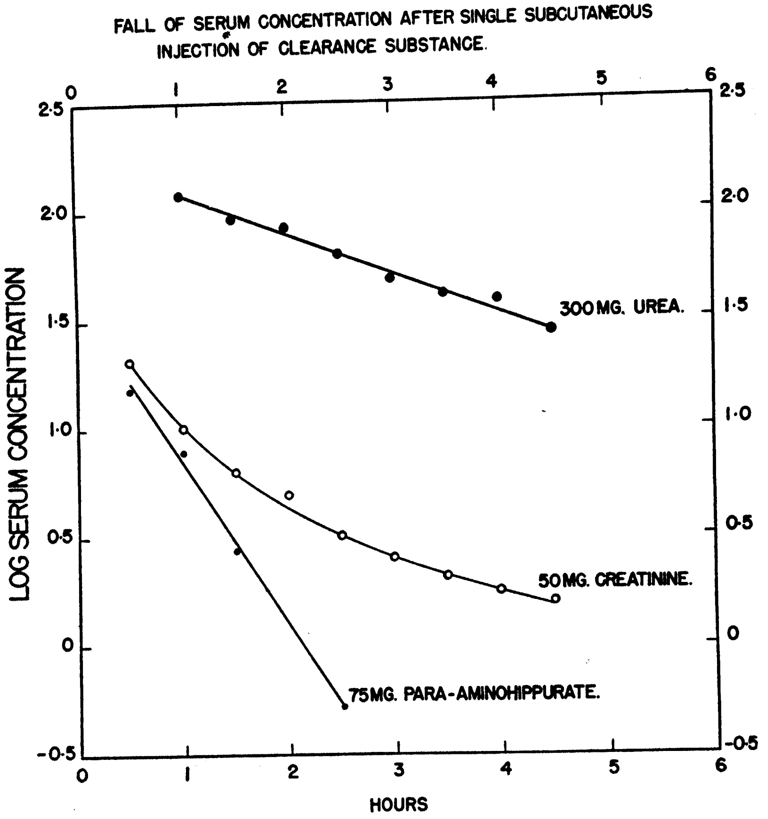

FIG. 1

TABLE 1. CLEARANCES IN THE NORMAL RAT

	CC/MIN/GRAMS KW			CC/MIN/100 GRAMS BW	
	'Undisturb.'	'Tailcut.'	% of undist.	'Undisturb.'	'Tailcut.'
Creatinine	2.27	1.43	−37	1.49	0.915
Urea	1.06	0.669	−37	0.703	0.458
PAH	4.82	4.17	−14	3.38	2.63

COMMENT

Friedman and Livingstone (6) determined diodrast and inulin clearances in rats. Their procedure utilized subcutaneous injection, anesthesia and withdrawal of 1.5 cc. of blood midway in the collection period. Before starting collection, they ligated the urethra without anesthesia, a procedure which presumably is painful. Even brief anesthesia results in temporary suppression of urine formation (7). The mean inulin clearance they obtained was 0.27 cc/min/

100 grams BW, which is only 39 per cent of the values obtained by Meyer Friedman and Braun Menendez at high urine volumes.

TABLE 2. CREATININE CLEARANCES

NO. RATS IN GROUP	MEAN GRAMS BW	MEAN MGM./KW	MEAN URINE VOL. CC/MIN/ GRAMS KW	MEAN CREAT. EXCRETION MGM/MIN/ GRAMS KW	MIDPOINT SERUM CREAT. MGM. %	CLEARANCE CC/MIN/GRAMS KW
a. 'Undisturbed'						
12	164	1001	.050	.222	10.00	2.22
12	166	1221	.038	.205	8.40	2.44
12	167	1117	.034	.136	7.59	1.79
12	168	1018	.031	.154	7.50	2.05
12	148	948	.028	.127	7.45	1.70
11	168	1212	.036	.168	6.68	2.52
12	164	1094	.027	.116	5.62	2.07
11	171	1063	.044	.110	4.70	2.34
12	143	1003	.033	.0660	4.05	1.63
11	168	1122	.040	.0957	3.50	2.74
10	167	1155	.043	.0997	3.26	3.07
11	166	1157	.042	.0840	3.25	2.59
12	165	1040	.054	.0754	3.21	2.35
11	144	938	.059	.0800	2.92	2.75
12	163	1057	.032	.0540	2.74	1.97
12	169	1128	.055	.0817	2.63	3.11
12	148	953	.058	.0730	2.50	2.92
11	173	1033	.050	.0417	2.37	1.76
12	170	1119	.052	.0438	2.28	1.92
12	162	1018	.044	.0385	2.05	1.88
12	173	1054	.055	.0384	2.01	1.92
b. 'Tailcutting'						
8	176	1037	.024	.204	14.0	1.45
7	179	1088	.020	.194	13.4	1.45
6	181	1162	.018	.204	11.8	1.74
10	186	1178	.023	.0663	5.06	1.31
8	186	1047	.022	.0589	4.90	1.20
8	181	1236	.020	.0657	4.72	1.39
5	180	1102	.029	.0570	4.68	1.22
7	177	1195	.028	.0608	4.22	1.44
6	184	1279	.031	.0564	4.09	1.38
6	171	1131	.021	.0697	4.07	1.71
5	190	1321	.030	.0663	4.02	1.65
7	174	1217	.023	.0455	3.80	1.20
5	170	1150	.025	.0532	3.76	1.41
8	360	2059	.032	.0386	2.77	1.39

Dicker and Heller (8) determined diodone and inulin clearances in the rat. They administered fluid by stomach tube, as well as by subcutaneous injection. Using a 10- to 25-minute collection period, they were obliged to deal with very

TABLE 3. UREA CLEARANCES

NO. RATS IN GROUP	MEAN GRAMS BW	MEAN MGM. KW	MEAN URINE VOL. CC/MIN/GRAMS KW	MEAN UREA EXCRETION MGM/MIN/GRAMS KW	MIDPOINT SERUM UREA MGM. %	CLEARANCE CC/MIN/GRAMS KW
a. 'Undisturbed'						
10	163	1029	.055	1.28	126	1.02
12	164	1001	.050	1.15	119	0.963
12	148	948	.028	0.898	112	0.812
12	166	1221	.038	0.940	108	0.870
12	168	1018	.031	0.938	104	0.901
11	168	1212	.036	0.915	104	0.880
12	164	1094	.027	0.828	104	0.795
12	167	1117	.034	0.840	94.9	0.885
11	171	1063	.044	0.845	77.7	1.09
12	143	1003	.033	0.649	69.7	0.930
11	166	1157	.042	0.789	68.2	1.16
10	167	1155	.043	0.922	64.1	1.44
11	168	1122	.040	0.768	58.8	1.31
12	165	1040	.054	0.776	58.8	1.32
11	144	938	.059	0.870	56.2	1.55
b. 'Tailcutting'						
6	171	1131	.021	0.583	79.8	0.731
5	190	1321	.032	0.683	78.7	0.868
6	184	1279	.031	0.423	70.5	0.600
5	170	1150	.025	0.392	68.4	0.572
7	174	1217	.023	0.386	67.5	0.572

TABLE 4. PAH CLEARANCES

NO. RATS IN GROUP	MEAN GRAMS BW	MEAN MGM. KW	MEAN URINE VOL. CC/MIN/GRAMS KW	MEAN PAH EXCRETION MGM/MIN/GRAMS KW	MIDPOINT SERUM PAH MGM. %	CLEARANCE CC/MIN/GRAMS KW
a. 'Undisturbed'						
10	149	1065	.078	0.377	7.96	4.74
6	156	1152	.029	0.381	7.50	5.08
5	159	1104	.044	0.359	7.18	4.99
6	153	1115	.033	0.357	7.13	5.00
10	162	1043	.034	0.298	6.88	4.33
10	163	1120	.034	0.278	5.82	4.78
b. 'Tailcutting'						
8	176	1037	.024	0.308	7.68	4.02
7	179	1088	.020	0.275	7.04	3.90
6	181	1162	.016	0.287	6.15	4.67
7	181	1178	.011	0.224	5.77	3.90
7	181	1218	.008	0.212	4.86	4.35

small quantities of urine, thus magnifying errors of collection. They dispensed with midpoint blood collection, using an end serum concentration, and obtained a mean inulin clearance of 0.351 cc/min/100 grams BW, which did not change with urine flow.

Meyer Friedman (9) obtained creatinine, inulin and PAH clearances in the rat. He used a procedure similar to our 'tailcutting' conditions, and obtained clearances which varied with the urine flow, the maximum figure for inulin clearance being 0.685 cc/min/100 grams BW. Similar results have been obtained by Braun Menendez and Chiodi (10).

In a later communication, Dicker and Heller (11) have commented on the findings of Meyer Friedman. They believe that the rate of glomerular filtration in the rat does not vary with urine flow except under extreme conditions, when it probably reflects reduced renal blood flow, and attribute Meyer Friedman's results to shock. However, this fails to explain why Meyer Friedman's highest inulin clearances, obtained at the highest urine flow, were twice as high as those of Dicker and Heller.

Most recently, S. Friedman and others (12), using an end blood and urine collected for 50 minutes after a single subcutaneous injection, obtained inulin and PAH clearances. Their results are expressed in terms of body surface, according to Meeh's formula, as an approximation for kidney size. It is difficult to compare their figures with previous findings in terms of body weight, or our own in terms of kidney weight itself. Furthermore, a correction applied for kidney size, in terms of surface area, must consider sex, as there is a sex difference of significant magnitude between kidney sizes at a given body weight. Kidney weight varies with the 0.72 power of body weight in males and the 0.65 power of body weight in females (13).

S. Friedman was able to maintain a steady serum concentration of PAH over an hour period after a single subcutaneous injection. This is contrary to the findings of Newman and of others who have studied the fall in serum concentration after single injections of a clearance substance (14). It has been our experience that PAH is absorbed from the site of injection and is cleared from the blood stream with such speed that, following a single subcutaneous injection of 75 mgm. in a rat weighing 170 grams, the serum concentration reaches a peak in less than 30 minutes. After that the serum concentration falls very rapidly; 90 minutes later the concentration has decreased from 15 to 1 mgm. per cent. We repeated such determinations, using a 50 mgm. dose and the conditions described by S. Friedman and others, but were unable to duplicate their results. After following their method of injection, we exsanguinated groups of animals at 30 and at 50 minutes, and found that the serum concentration which was 13.1 mgm. per cent at 30 minutes had fallen to 5.41 mgm. per cent at the end of 50 minutes. We believe that the values for PAH clearance given by these authors are higher than the true values because of the error introduced by using only endpoint bloods.

Criticism for using only endpoint bloods also applies to the work of Dicker and Heller. If endpoints alone are used, it is not valid to assume that the drop in serum concentration has been inconsequential. It is necessary to obtain an estimated midpoint serum concentration by extrapolation along a line relating log concentration to time, obtained under conditions precisely similar to those used for the clearance determinations, using very large numbers of rats.

The logarithmic rate at which the serum concentration of urea and PAH fall after 30 minutes suggests that absorption is proceeding at a steady rate by that time, so that excretion proceeds according to the assumptions of Newman, Bordley and Winternitz (14). The fact that creatinine does not behave in precisely the same manner suggests that it is less rapidly and completely absorbed from the injection site, or that the assumptions of Newman and others do not hold for this substance.

In attempting to explain the difference between urea and creatinine clearances obtained by 'tailcutting' and 'undisturbed' methods, our first thought was that the renal plasma flow might be diminished as a result of diminished total circulating blood volume. However, blood volumes estimated from 'drawn blood' volumes and by actual determination (table 5) were found to be normal.

TABLE 5. 'TAILCUTTING' BLOOD VOLUME DETERMINATIONS

WEIGHT, GRAMS	CC. PLASMA VOL.	CC. BLOOD VOL.	CC. NORMAL PLASMA VOL.	CC. NORMAL BLOOD VOL. (4)
142	5.52	9.08		
144	5.92	9.85		
140	7.06	11.50		
146	6.65	10.50		
138	6.75	11.00		
146	6.00	9.18		
146	5.61	9.07		
140	5.67	9.12		
144	5.77	8.77		
Mean..143	6.11	9.79	5.78	10.00

As can be seen in table 4, there appears to be a difference between the 'undisturbed' and 'tailcutting' PAH clearances of 14 per cent, presumably due to a difference in the renal plasma flow.

It has been shown that the renal circulation is most sensitive in responding to emergency situations, and is independent of the general circulation in large part (15, 16). The difference in renal plasma flow between the methods mentioned above must be the result of an alteration in the local renal hemodynamics which is not associated with a change in the general blood volume. But the degree by which renal plasma flow is diminished, 14 per cent as indicated by the PAH clearance, is not adequate to explain the 37 per cent fall in creatinine and urea clearances. It seems reasonable to suppose that additional changes in the internal renal hemodynamics, such as a change in filtration pressure, from alteration in the tonus of afferent and efferent arterioles, must be involved.

It is evident from our tables that urine flow, as well as clearance determinations, was depressed by the conditions of 'tailcutting'. However, inspection will also reveal that, within each set of conditions, the clearances did not bear any relation to urine flow. This would seem to confirm partially the idea of Dicker and Heller that the clearances are altered only with extreme changes in urine flow, which reflect reduced renal plasma flow. However, as demonstrated above, diminution of renal plasma flow is not the whole explanation. Urine flow is a complex resultant of glomerular filtration and tubular reabsorption of water. It is more reasonable to suppose that reabsorption of water, along with other components of renal function, is affected by the changes in both plasma flow and filtration pressure that result from alterations in the conditions of determination.

Consideration of the various ways in which renal clearances have been performed on the rat leads inescapably to the conclusion that no method can be considered entirely satisfactory. The clearance values already reported by different authors vary by more than 100 per cent. Such variability cannot arise from errors in the chemical determinations. It seems very probable that, the more the rat is disturbed by manipulations necessary to make urine and blood collections, the more the clearance values are depressed from their values in the undisturbed animal. We have shown that the decrease in clearance values associated with 'tailcutting' is only in part explained by decrease in the rate of plasma flow through the kidneys. The possibility that there is a decrease in the filtration fraction is now under investigation.

In spite of the fact that 'tailcutting' conditions have been shown to depress clearance values, these conditions give us less variation than is to be found in the 'undisturbed' clearance values. Our 'undisturbed' creatinine clearances have a coefficient of variability of 20 per cent, while the coefficient of variability of the 'tailcutting' clearances is only 12 per cent. We think that this is a consequence of errors introduced by the necessity of calculating extrapolated midpoint serum concentrations from an endpoint specimen. In view of the imperfections associated with any practical set of conditions, it would seem wisest to use that set which permits determination with the least degree of variability, if the purpose is to analyze by simultaneous determinations the mechanism involved in the excretion of clearance substances. On the other hand, if the purpose is to study the relations between renal function and other functions of the body, it would seem more desirable to use conditions that produce the least depression of renal function, even though greater variability is incurred.

SUMMARY

1. Renal clearances of creatinine and urea have been determined under two sets of conditions. 'Tailcutting' appears to depress renal clearances through effects upon both external and internal hemodynamic relations of the kidney.

2. Steady serum concentration of clearance substances cannot be maintained by a single subcutaneous injection. Consequently, use of a single endpoint serum concentration introduces large errors, unless the midpoint concentration is approximated from actual determinations of the rate at which the serum concentration falls after a single injection.

3. Use of a 'tailcutting' method is proposed for certain purposes, in spite of

the resulting depression of clearance values, because more accurate determination of the midpoint serum concentration reduces the variability of clearance determinations.

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REFERENCES

1. Barrett, E., and T. Addis. J. Clin. Invest. **26:** 875, 1947.
2. Addis, T. J. Lab. Clin. Med. **10:** 402, 1925.
3. Smith, H. W., N. Finkelstein, L. Aliminosa, B. Crawford and M. Graber. J. Clin. Invest. **24:** 388, 1945.
4. Lippman, R. W. Proc. Soc. Exper. Biol. & Med. **66:** 188, 1947.
5. Lippman, R. W. This Journal **151:** 211, 1947.
6. Friedman, S. M. and C. A. Livingstone. This Journal **137:** 564, 1942.
7. Heller, H. and F. H. Smirk. J. Physiol. **76:** 283, 1932.
8. Dicker, S. E. and H. Heller. J. Physiol. **103:** 449, 1945.
9. Friedman, M. This Journal **148:** 387, 1947.
10. Braun Menendez, E. and H. Chiodi. Rev. Soc. Argent. de Biol. **22:** 314, 1946.
11. Dicker, S. E. and H. Heller. Science **106:** 127, 1947.
12. Friedman, S. M., J. R. Polley and C. L. Friedman. This Journal **150:** 340, 1947.
13. MacKay, L. L. and E. M. MacKay. This Journal **83:** 196, 1927.
14. Newman, E. V., J. Bordley, iii and J. Winternitz. Bull. J. Hopkins Hosp. **75:** 253, 1944.
15. Chasis, H., H. A. Ranges, W. Goldring and H. W. Smith. J. Clin. Invest. **17:** 683, 1938.
16. Corcoran, A. C. and I. H. Page. This Journal **126:** 354, 1939.

VOLUME MEASUREMENT OF THE ISLETS OF LANGERHANS AND THE EFFECTS OF AGE AND FASTING[1]

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There is good evidence that the islets of Langerhans in the pancreas constitute a tissue with at least one special function, namely, the production of insulin. It is important to consider this islet tissue as a whole, to know with as much precision as possible the total bulk of the endocrine pancreas and how its volume changes under experimental conditions. A number of studies in this field have been undertaken, using representative fixed and stained tissue sections (1–5). The method which will be described in this paper uses fresh, intravitally stained material and, we believe, can be employed conveniently to study factors influencing the growth or new formation of islet cells.

MATERIALS AND METHODS

Rats are injected intravenously with a 2.5 per cent solution of neutral red in physiological saline (1 cc/200 grams of body weight). This gives a differentiation between islets and acini as described by Bensley and others (6, 7). The pancreas is removed 45 minutes after the injection, is weighed and its specific gravity determined. The pancreas is kept cold and cut into small pieces. These pieces are mounted in 10 per cent formol-saline solution between lantern-slide plates. A photographed grid on one of the plates helps to ensure that islets will neither be overlooked nor measured twice. The two plates are pressed together with clips and kept at 0°C. for 15 minutes, then quickly frozen in a mixture of solid CO_2 and alcohol and placed in a thermos flask over solid CO_2 for storage. Under these conditions the preparations keep for at least one week and probably much longer. Before use, the preparation is allowed to thaw. The plate is placed on the stage of a microprojector and moved from field to field at table level by cable controls. The image is projected onto a movable platform which holds a planimeter. The area of the piece of pancreas is given by the total number of fields it contains. The area of the islets is measured by a planimeter procedure. The planimeter rests on a movable platform which slides on a glass plate (fig. 1). The special point of the planimeter rests in a notch in a lucite strip, *A*. This notch is the constant reference point. The reference point is moved up to the edge of an islet and its outline is traced with the planimeter point. This gives a permanent record on a paper roll, *B*, which is backed by a carbon-paper strip. After one islet is traced, the knob *C* is turned. This moves the paper and records the islet count, *D*. The reference point is then moved up to the next islet and the procedure repeated. By keeping a constant reference point for the planimeter and moving it up to the next islet by moving the whole platform, the planimeter is made to add the islet areas. When all the islets in one field are measured, the field is changed and its number recorded by a counter. Hence the procedure gives the total area of the islets, the total area of the piece, the number of islets

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[1] A preliminary report of this work appeared in Federation Proc. 6: 119, 1947.

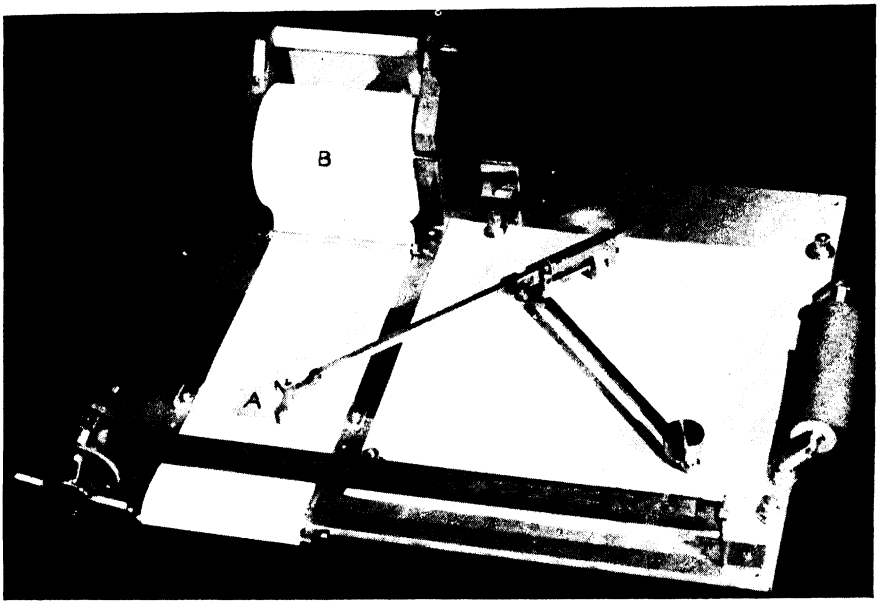

FIG. 1

TABLE 1[1]

ISLET AREA		TOTAL AREA		RATIO OF INITIAL TO FINAL AREAS	
Light pressure	Increased pressure	Light pressure	Increased pressure	Islets	Total
4.2	5.0	56.9	65.3	0.84	0.87
1.81	2.11	61.5	72.5	0.95	0.85
8.98	12.55	84.82	107.05	0.72	0.79
0.19	0.32	26.89	43.74	0.59	0.61
0.41	0.49	41.09	53.75	0.84	0.76
0.25	0.31	53.84	69.05	0.81	0.78

[1] The areas given here are in sq. cm. They are the areas of the projected images which are the actual areas magnified 79.6 diameters.

and a permanent record of the islet outlines. The latter is useful in determining the spread in islet size.

Provided that the pressure is sufficient to compress the islets into discs, the ratio of the islet area to the total pancreas area does not alter significantly with a further increase in pressure. Table 1 gives values for representative islets and corresponding acinar areas before and after increasing the pressure on the glass plates. The fading of the stains with the repeated examination makes this procedure technically difficult. For this reason the number of islets examined is limited and the total areas measured are small. The error consequently is large, but the results indicate that the acinar and islet areas increase relatively to the same extent when large changes in pressure are applied to the plates.

The $\frac{\text{islet volume}}{\text{total pancreas volume}}$ ratio is proportional to the $\frac{\text{islet area}}{\text{total pancreas area}}$ ratio. These ratios should be the same if the islets extend from glass to glass between the plates in the preparations. Examination of paraffin-embedded cross-sections of the preparations showed, however, that the majority of the islets did not extend through the full thickness of the preparation. Hence it was necessary to establish a correction factor relating the average islet thickness to the average thickness of the preparation.

The correction factor is now obtained, using fresh preparations. These are made in the usual way. They are then peeled off the glass plates and stacked like coins, using thick warm gelatine as an adhesive agent. The stack is then frozen and serial cross-sections are cut through it. The sections are then placed in the microprojector and tracings of the islets and of the corresponding portions of the whole thickness of each piece of pancreas are made. The total length of the islets and the total area of the islets in these tracings are then obtained and from this the average islet thickness is estimated. The average thickness of the preparation is also determined. The correction factor is the ratio of $\frac{\text{the average islet thickness}}{\text{the average thickness of the preparations}}$.

RESULTS

In figure 2 the correction factors obtained for rats of different weights are shown.[2] For all practical purposes the correction factor is the same for rats weighing from 50–300 grams. The average factor for 12 rats is 0.40 s ± 0.05. The regression coefficient (slope of the curve between the body weight and the correction factor) is 0.000156 ± 0.00018, i.e., the regression coefficient does not differ significantly from zero. Hence the correction factor can be considered as independent of body weight in the weight range studied. Table 2 gives the collected data for the various experiments. This shows the consistency of the results and the effect of age and of fasting on islet counts and islet volume. In figure 3 the effect of age (body weight) on islet volume is shown more clearly. It will be evident that the islet volume increases with age and also that the consistency of the results is good. Figure 4 shows that the number of islets increases with age. Hence part at least of the increase in islet volume with age is due to the development of new islets. When the islet volume is related to the body

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[2] We are indebted to our colleagues Mr. B. Kinash and Dr. F. Bryans for the determination of the correction factor.

We wish to thank Prof. C. H. Best for his interest and encouragement in this work. It is a pleasure also to acknowledge that this study was largely supported by a grant from the National Research Council of Canada.

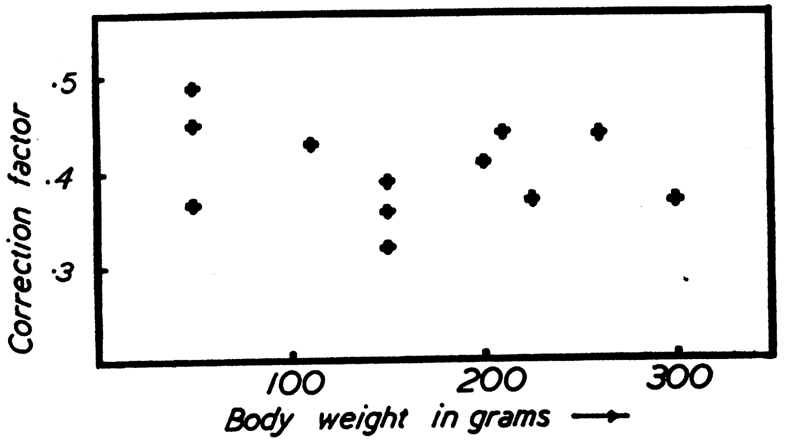


FIG. 2

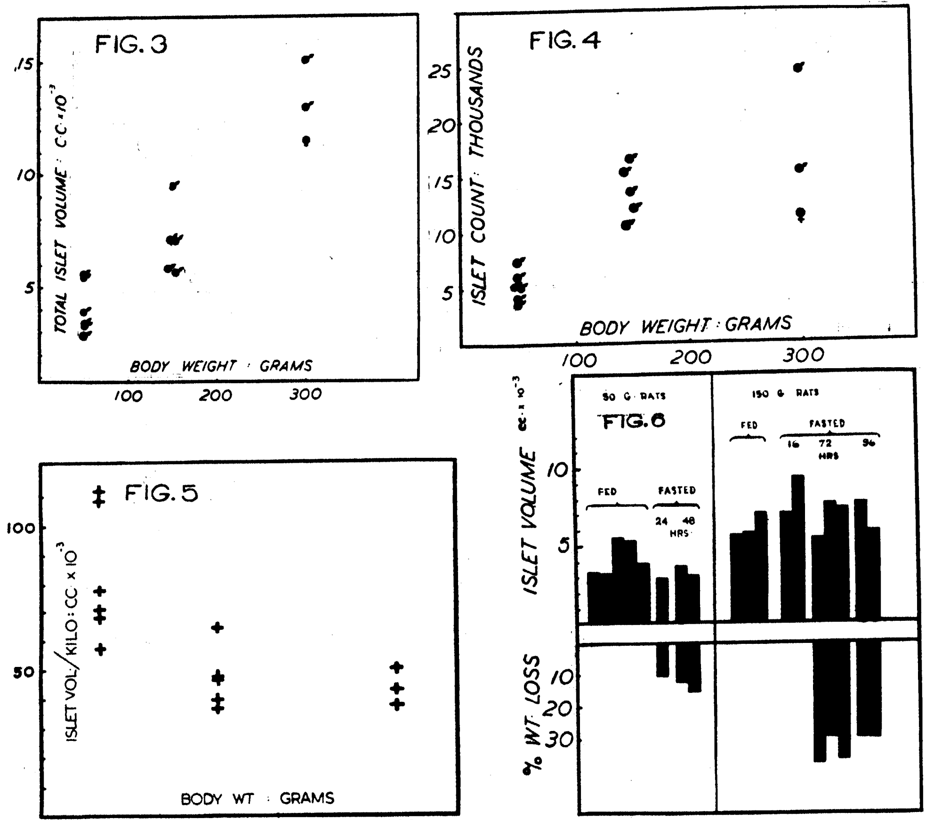
TABLE 2

RAT WT.	SEX	NO. ISLETS	PANCREAS WT.	IMAGE AREAS: ISLET / TOTAL PANCREAS	ISLET x 100 / TOTAL PANCREAS (CORRECTED)[1]	TOTAL ISLET VOL. CC. x 10^{-3}	STATE
grams			*mgm.*				
50	♦ M	4,153	294	0.0301	1.20	3.4	Fed
50	M	3,634	315	0.0283	1.13	3.3	Fed
50	M	5,103	308	0.0492	1.97	5.6	Fed
50	M	5,150	292	0.0497	1.99	5.4	Fed
50	M	6,088	295	0.0353	1.41	3.9	Fed
50	M	7,468	301	0.0261	1.04	2.9	Fed
48–45.6	M	10,557	231	0.0365	1.46	3.1	Fasted 24 hrs.
53–43.4	F	8,362	269	0.0377	1.51	3.8	Fasted 48 hrs.
53–42	F	7,742	260	0.0334	1.34	3.2	Fasted 48 hrs.
153	M	12,198	695	0.0214	0.86	5.7	Fed
145	M	15,434	640	0.0245	0.98	5.8	Fed
150	M	13,700	543	0.0346	1.38	7.1	Fed
150	M	16,588	576	0.0334	1.34	7.1	Fasted 16 hrs.
146	M	10,552	469	0.0540	2.16	9.5	Fasted 16 hrs.
145–90	M	15,822	457	0.0314	1.26	5.4	Fasted 72 hrs.
140–90	M	12,546	525	0.0402	1.61	7.4	Fasted 72 hrs.
150–105	M	8,502	498	0.0422	1.69	7.8	Fasted 72 hrs.
150–105	M	10,516	460	0.0458	1.83	7.7	Fasted 96 hrs.
150–105	M	9,374	382	0.0425	1.70	6.0	Fasted 96 hrs.
155–112	M	11,000	460	0.0389	1.56	7.2	Fasted 96 hrs.
300	M	15,490	958	0.0377	1.51	13.0	Fed
300	F	11,476	972	0.0314	1.26	11.5	Fed
300	M	24,686	1223	0.0334	1.34	15.1	Fasted 16 hrs.

In the heavier weight groups, the animals fasted for 16 hours are considered to be normal animals.

[1] Correction factor 0.40 s ± 0.05.

weight, as in figure 5, it becomes evident that the islet volume per unit of body weight decreases with age. Rats were fasted for varying periods of time and the weight was recorded. Figure 6 gives the percentage weight loss in the lower columns and the islet volumes in the upper columns for the fasted rats. Even when there was a reduction of 30 per cent in the body weight, the total islet volume remained unchanged. It was felt that a short period of fasting improved the consistency of the results. Referring to table 2, it can be seen that no consistent change in the number of islets results from fasting under the conditions of these experiments.



FIGS. 3–6

DISCUSSION AND CONCLUSIONS

A method has been described for the estimation of the volume of the islets of Langerhans in fresh tissue preparations. Without a correction factor, the values obtained are proportional to the islet volumes. With a correction factor, more nearly absolute values for the islet volume may be obtained. A method for obtaining the correction factor has been outlined. The factor is relatively constant for normal rats ranging in weight between 50 and 300 grams.

From the data presented it is evident that the volume of the islets and the islet count increase with age. If, however, the islet volume or islet count is related to body weight, it is found that the volume or count per unit of body weight decreases with age. Previous work showed that a similar increase in the insulin content of the pancreas occurs with age in the rat (8). Using values for the insulin content of pancreas and for the islet volumes for rats of different ages it can be computed that the insulin content per unit of islet volume does not change greatly with age. Such a calculation yielded approximate values ranging from 150 to 200 units of insulin per cc. of islet tissue for a body weight range of from 50 to 300 grams. This indicates that less than one per cent of the islet is insulin.

Fasting for periods sufficient to cause a reduction of approximately 30 per cent in body weight had no appreciable effect on the islet volume or islet count. The latter supports the earlier report of Bensley (6). Since previous work indicated that fasting of this degree led to a marked reduction in the insulin content of the pancreas (9), it becomes evident that fasting leads to a reduction in the concentration of insulin per unit volume of islet tissue. This conclusion is in harmony with the finding that fasting reduces the degree of granulation of the beta cells of the islets of Langerhans.

SUMMARY

1. A method of estimating the volume of the islets of Langerhans in fresh pancreas is outlined.

2. Using this method, it was found that the volume of the islets in rats increases with age.

3. The islet count increases with age.

4. Fasting does not lead to any consistent change in the islet count.

5. Fasting does not appreciably alter the islet volume.

6. These findings are discussed and are related to the results of previous studies on the insulin content of pancreas.

REFERENCES

1. Ogilvie, R. F. Quart. J. Med. **6**: 287, 1937.
2. Richardson, K. C. and F. G. Young. J. Physiol. **91**: 352, 1937.
3. DeWitt, L. M. J. Exp. Med. **8**: 193, 1906.
4. Susman, W. J. Clin. Endocrinol. **2**: 97, 1942..
5. Tejning, S. Acta Med. Scand.: supplement 198, 1947.
6. Bensley, R. R. Am. J. Anat. **12**: 297, 1911
7. Bensley, R. R. Harvey Lectures. **10**: 250, 1914–15.
8. Haist, R. E. and H. J. Bell. This Journal **141**: 606, 1941.
9. Best, C. H., R. E. Haist, and J. H. Ridout. J. Physiol. **97**: 107, 1939.

APPLICATION OF THE SATURATION PRINCIPLE TO THE ESTIMATION OF FUNCTIONAL HEPATIC MASS IN NORMAL DOGS

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Studies of renal function have demonstrated that the maximum tubular secretion or reabsorption rates obtained for certain solutes at plasma concentrations saturating the mechanisms concerned may be considered a measure of the active tubular mass (Tm) in respect to the substance employed (1). Similarly, measurement of the maximum excretion of a substance largely or exclusively secreted into the bile should give a corresponding estimate of the functional hepatic mass ('Lm') for the solute employed. The following report deals with studies on the maximum excretion of bromsulphalein by dogs under conditions of apparent saturation of the mechanisms concerned and is an extension of a previous communication (2).

PROCEDURE

The principle of the procedure for determination of hepatic excretory mass is a simple one previously suggested for application to studies of renal function (3). It avoids the necessity of making a quantitative collection of bile and may be applicable to a number of substances which are excreted or metabolized largely or exclusively by the liver.

If the substance to be excreted is intravenously infused at a rate so that a constant plasma concentration of sufficient magnitude to saturate the excretory mechanisms is obtained, then, providing the plasma concentration remains constant, the rate of infusion is equal to the rate of excretion. In actual experiments a correction may be made for observed rise or fall in plasma levels, provided that the nature of distribution of the substance in the body is known. In addition, correction must be made for extrahepatic disposal of the substance if such occurs. From the corrected infusion rate the maximum mass of substance excreted per unit time is readily calculated. The estimation of bromsulphalein excretion in man by infusion at a rate producing a constant blood level has been previously described in an abstract by Ingelfinger (4) and in more detail by Bradley *et al.* (5) in connection with the determination of hepatic blood flow.

In applying this principle to the excretion of bromsulphalein, no assumptions are made regarding the mechanisms of hepatic excretion of this dye other than that these are 'saturated' during the test at the plasma concentrations achieved (see below). Extrahepatic loss via the urine is readily measured and in any event in the case of dogs, it is an almost negligible fraction of the dye infused over a range of plasma levels considerably above the apparent saturation limit.

The correction applied to the infusion rate for a rise or fall of plasma concentration is made upon the basis that any dye entering the circulation when the plasma level is already above the saturation limit is distributed in the plasma only (5). The details of the means of further establishing this will be reported in a subsequent communication. Tentatively, it would appear that in dogs the correction may be made with sufficient accuracy by estimating the plasma volume from the hematocrit reading and a blood volume calculated as 9 per cent of the body weight, as is the case in the experiments reported below. The error involved in estimating the correction by such means decreases as the slope of the curve relating plasma concentration to time approaches zero.

EXPERIMENTAL

Dogs were used in the experiments. No anesthetic was employed other than novocaine occasionally used to infiltrate sites of subsequent venepuncture. It was found, however, that the animal had to be sufficiently trained to submit to the procedures quietly. The actual measurement of maximum hepatic excretion of bromsulphalein was made as follows.

A needle was inserted into a saphenous vein. This was connected by a short piece of tubing to a 50 cc. syringe containing the bromsulphalein solution to be injected at a constant rate. The syringe was seated in the slot of an apparatus equipped with a threaded rod which was connected by a worm gear to an electric motor and so arranged that the threaded rod drove the plunger of the syringe at any rate desired. Alternately the gear mechanism was operated by hand.

Infusion of the bromsulphalein (Hynson, Westcott and Dunning, sterile solution, 50 mgm. dye/cc., buffered to pH 7.4 with disodium phosphate and diluted with sterile saline to whatever strength desired) was started at a rate estimated to represent the maximum rate of excretion. For normal dogs of an average size (10 to 15 kgm.) this is about 0.45 mgm/kgm. body wt/min. As soon as the infusion was started a priming dose of the dye amounting to from 15 to 30 mgm/kgm. body wt. was injected, employing either some other accessible vein or the already inserted needle connected to the infusion apparatus. In some instances the priming dose was given first, with constant infusion following immediately. After allowing a period of 10 to 15 minutes to elapse to establish mixing and saturation, blood specimens of 5 cc. each with or without anti-coagulant added were taken at intervals of 5, 10 or 15 minutes from the femoral vein of the leg opposite the site of infusion. The constant injection was continued for periods of 30 minutes to an hour or more from the time of the withdrawal of the first blood specimen. Shorter infusions may be found to be sufficient, although to date it has been noted that in some dogs the infusion must be maintained for from 20 to 40 minutes before the plasma concentration of the dye ceases to exhibit considerable fluctuation. In most instances the animals were catheterized so that urine was collected at intervals and renal loss of the dye exactly measured.

Bromsulphalein in serum, plasma or urine was determined by the photoelectric procedure of Gaebler (6), which is quite precise even in the presence of moderate degrees of lipemia, hemolysis and icterus. The exact concentration of the infused dye solution was also determined by analysis, rather than by calculation from the degree of dilution of the stock solution. The hematocrit reading of a portion of the first blood specimen drawn was determined by the Wintrobe procedure, employing isotonic oxalate anticoagulant. Of the several formulations available for the calculation of the surface area of dogs (7), that of Meeh and Rubner was employed.

TABLE 1. 'Lm' (BROMSULPHALEIN); NORMAL DOGS

DOGS	WEIGHT	AVERAGE PLASMA BROMSULPHALEIN CONCENTRATION DURING TEST	Lm	
			Mgm/kgm/min.	Mgm/min./sq.M. surface area
	kgm.	*mgm. %*		
1	10.90	5.5–27.0	0.49 (av. of 5 det'ns.)	9.9 (av. of 5 det'ns.)
2	14.50	4	0.42	9.1
3	10.46	4	0.43	8.5
4	8.20	10	0.42	7.5
5	9.50	10	0.41	7.8
6	20.40	5	0.41	9.9
7	12.30	4	0.41	8.6
8	13.40	8	0.44	9.4
9	19.50	8	0.39	9.5
10	15.40	9	0.33	7.5
Average			0.44	9.1

TABLE 2. REPRODUCIBILITY OF 'Lm' (BROMSULPHALEIN)
(Dog No. 1)

DATE	AVERAGE PLASMA BROMSULPHALEIN CONCENTRATION DURING TEST	Lm		REMARKS
		Mgm/kgm/min.	Mgm/min/sq.M. surface area	
	mgm. %			
3/18	5.5	0.48	10.0	
3/25	17.0	0.47	9.9	
4/1	27.0	0.52	10.3	
4/4	25.0	0.48	9.6	
4/10	9.0	0.37	7.2	48 hours after CCl_4; 1 cc/kgm.
4/22	13.5	0.49	9.7	
8/21	7.0	0.42	9.3	

RESULTS

A typical experiment and the calculations involved are shown in figure 1. Table 1 summarizes the results of 14 tests on 10 normal dogs. It is seen that the maximum hepatic excretory rate for bromsulphalein ('Lm', bromsulphalein) is of the order of 9.1 mgm. per minute. Table 2 summarizes the results of repeated tests upon the same dog and includes the various plasma concentrations of dye attained in each experiment. In addition, the results of three tests after the oral administration of a small dose of carbon tetrachloride are given.

DISCUSSION

The 'Lm' (bromsulphalein) of normal dogs. A considerably larger series of animals must be studied in order to assess properly the spread of values to be anticipated in normal animals; however, these preliminary results indicate that the range will not be a large one. Similar results have been obtained by others employing the procedure described (8).

It may be noted that the 'Lm' for the dog on whom many tests were performed (table 2) is distinctly greater than that of most of the remaining animals reported. This may be due to physiological variability, as upon subsequent laparotomy this animal exhibited an unusually large liver. Sections from a lobe removed revealed entirely normal architecture. In addition, this animal was the most satisfactorily trained of the group studied and had received a diet containing large supplements of horse meat for some time, and these factors may be concerned. It was consistently observed that the excretion rate became slower and highly variable when the animals manifested hard struggling or excitement. The plasma levels of the dye, instead of rising or falling smoothly as the infusion progressed, exhibited marked fluctuations. With moderate restlessness the excretory rate may fall somewhat, which may account for the rather low values obtained for *dogs 4, 5,* and *10* (table 1). In the calculation of the 'Lm' it is only the initial and final plasma concentrations for the time period chosen which are employed in making the corrections for retention or extra excretion of dye. However, obtaining intermediate values permits an estimate as to whether or not conditions influencing excretion of the dye (probably hepatic blood flow) have been reasonably constant during the test.

In a few experiments in which light anesthesia was induced with pentobarbital, most of the excretory rates obtained were much lower than those observed in unanesthetized animals.

These values for 'Lm, bromsulfalein' obtained for dogs are considerably greater than the maximum excretory rate of approximately 3 mgm/sq. M/min. reported by Inglefinger (4) employing human subjects. We have applied the procedure described here to a series of normal patients and have found 'Lm' to be about 14. The reason for this discrepancy is not clear at the present time.

The serum concentration of bromsulphalein required for saturation. It is seen in table 2 that the maximum excretory rate remained essentially constant over a wide range of serum concentrations of dye, lending support to the assumption of saturation of the excretory mechanisms. The maximum level at which saturation is achieved has not been carefully ascertained as yet. Preliminary data make it appear that the saturation level is below 4 mgm. per cent plasma concentration of the dye. It is the priming dose which is largely responsible for the rapid attainment of saturation, and the analysis of certain data not reported here further indicates that in the conventional bromsulphalein test of liver function the rate of the initial rapid disappearance of most of the dye from the blood stream has no relation to functional hepatic excretory mass but is more nearly a function of the mass of reticulo-endothelial tissue (5, 9–11). Serum concentrations well above the saturation limit have always been obtained in dogs with priming doses of dye of 15 to 20 mgm/kgm. body weight. We have not observed a diminution in maximum excretory rate with rising plasma dye concentrations such as has been reported to occur in case of perfusion of isolated fish livers (12).

Sensitivity of the test. Data upon the correlation between reduction of 'Lm' as measured by the procedure described and that produced by partial hepatectomy and experimental liver damage caused by hepatotoxic agents will be reported subsequently. In the single experiment of this nature reported above (table 2), a small but distinct reduction in 'Lm' was observed two days after the administration of carbon tetrachloride. The animal had not been ill and the quantity of carbon tetrachloride employed produced no symptoms at any time other than the immediate ataxia and depression during absorption of the drug. Fourteen days later the excretory rate was essentially normal again.

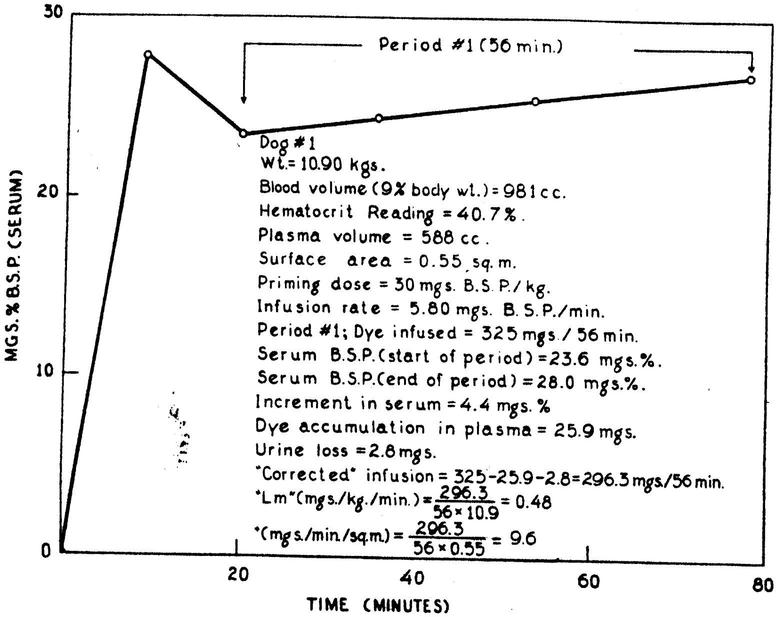

FIG. 1. LIVER MAXIMUM, bromsulphalein (= 'LM', bromsulphalein); dog

Toxicity of bromsulphalein. No evidence of toxicity resulting from infusions of the amounts of dye required has been observed with the exception of some local irritation of the vein employed for infusion. This in some instances has been followed by thrombosis of a small segment. This may be minimized by increasing the dilution of the solution infused. Temperature, pulse, respiration and blood pressure do not significantly alter during the infusion or afterwards, providing the infusion and priming solutions are *freshly* prepared.

Extrahepatic disposal of bromsulphalein. A rather large fraction of the initial priming dose of the dye is quickly removed from the vascular compartment probably by cells of the reticulo-endothelial system, until this means of disposal is saturated. Subsequently no further uptake of the dye occurs by this means or else the rate of transfer of the dye from this system to the liver cells attains a constant maximum rate (unpublished observations). At maintained plasma levels of 5 to 15 mgm. per cent, excretion via the urine was found to be only a small quantity, ranging from 1 to 4 per cent of the amount which had been infused during the period. At very high plasma levels the fraction escaping into the urine appears to increase considerably. At the plasma levels employed in the experiments reported here, no dye appears in extravascular, extracellular fluid. Thus hypertonic glucose administered intraperitoneally at the beginning of an experiment and withdrawn later was found to be essentially free of the dye. There is no escape via the salivary route nor does the dye appear in the cerebrospinal fluid. These findings agree with those previously reported by Bradley *et al.* (5) as obtaining in man.

SUMMARY

A technique for determination of the maximum rate of hepatic excretion of bromsulphalein ('Lm' bromsulphalein) has been described. It is believed that this rate obtained under conditions of saturation of the mechanisms involved should be proportional to the functional hepatic mass in respect to the substance excreted. The values of 'Lm' for a small series of normal dogs ranged from 7.5 to 10.3 mgm. min/sq. M. surface area. The value was found to be rather constant and reproducible in the same animal.

REFERENCES

1. Smith, H. W. The Physiology of the Kidney. Oxford University Press, 1938.
2. Mason, M. F., G. Hawley, W. Lewallen and H. G. Montgomery. Federation Proc. **6**: 1, 1947.
3. Earle, D. P. and R. W. Berliner. Proc. Soc. Exper. Biol. Med. **62**: 262, 1946.
4. Ingelfinger, F. J. J. Clin. Invest. **25**: 927, 1946.
5. Bradley, S. E., F. J. Ingelfinger, G. P. Bradley and J. J. Curry. J. Clin. Invest. **24**: 890, 1945.
6. Gaebler, O. Am. J. Clin. Path. **15**: 452, 1945.
7. Cowgill, G. R. and D. L. Drabkin. This Journal **81**: 36, 1927.
8. Anderson, C. E., R. G. Gale and C. S. Robinson. Personal communication; results to be published.
9. Wirts, C. W. and A. Cantarow. Am. J. Digest. Dis. **9**: 101, 1942.
10. Mills, M. A., and C. A. Dragstedt. Proc. Soc. Exper. Biol. Med. **34**: 228, 1936.
11. Klein, R. I. and S. A. Levinson. Proc. Soc. Exper. Biol. Med. **31**: 179, 1933.
12. Haywood, C., V. C. Dickerson and M. C. Collins. J. Cell. Comp. Physiol. **25**: 145, 1945.

ARTERIO-VENOUS ANASTOMOSES IN LIVER, SPLEEN, AND LUNGS[1]

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Arterio-venous anastomoses have been observed in various organs by some of the older anatomists. The literature on this subject to 1939 has been reviewed by Clara (1), who listed the nose, ear, glomus coccygium, ovaries, submandibular salivary gland, kidney, stomach, small intestine, mesentery, lymph nodes and the brain as sites of arterio-venous anastomoses. Among those organs listed by Clara there is no mention of studies of the liver, spleen or lungs. Furthermore, no unequivocal demonstration of arterio-venous anastomoses in these organs was found in the literature available to us.

In previous reports from this laboratory, arterio-venous anastomoses were demonstrated in the heart (2) and kidneys (3) by a technique involving the intra-arterial injection of glass spheres of known diameters into these organs. If glass spheres of diameters many times greater than the average diameter of capillaries can be recovered from the venous circulation of an organ after they are injected into the arterial circulation of that organ, then those spheres must have passed through channels which by-pass the capillaries. Also, since the diameters of the spheres are known, the minimum size of these anastomotic channels is indicated. It was the purpose of the present study to determine by this method whether or not arterio-venous, noncapillary anastomoses exist in the liver, spleen or lungs of living animals and, if so, the approximate size of these channels.

METHOD

The experiments were performed on anesthetized rabbits, cats and dogs. Glass spheres[2] ranging in diameter from 10 to 440 micra were suspended in saline and injected at physiologic pressure with an Asepto syringe through a 16-gauge needle into a large afferent blood vessel of the organ. Spheres carried into the venous circulation from the perfused organ were recovered in other organs and vessels which trap and collect them. Further details for the individual experiments are presented below. The animals were killed with sodium pentobarbital or potassium chloride within 30 seconds after injection, and the spheres were recovered by digestion with hot 30 per cent alcoholic potassium hydroxide. After digestion was complete, the residue was washed with water by decantation at five-minute intervals until clear solutions were obtained. The spheres were

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[1] Endowed by grants from the Blanche May Selden and Beaumont Trust Funds.

[2] Glass spheres were obtained from Prismo Products, 1211 Architects Building, Los Angeles, Calif.

removed from the beaker to glass slides with a rubber policeman, and their diameters measured under low power with a calibrated reticule in the eyepiece of the microscope.

Liver. Eleven rabbits weighing from 5 to 10 pounds were anesthetized with ether, and the portal vein was exposed by opening the abdomen. The suspension of glass spheres was injected into the portal vein within 5 mm. of its entrance into the liver. The thoracic cavity was opened immediately after injection and the lungs were removed. Spheres were recovered from the lungs in 10 of 11 rabbits (table 1). The maximum diameter of glass spheres recovered varied from 60 to 180 micra.

TABLE 1

ANIMAL	ORGAN STUDIED	INJECTION SITE	TISSUE IN WHICH SPHERES WERE RECOVERED	DIAMETER OF LARGEST SPHERE RECOVERED
				micra
Rabbit 1	Liver	Portal vein	Lungs	60
Rabbit 2	Liver	Portal vein	Lungs	80
Rabbit 3	Liver	Portal vein	Lungs	70
Rabbit 4	Liver	Portal vein	Lungs	180
Rabbit 5	Liver	Portal vein	Lungs	150
Rabbit 6	Liver	Portal vein	Lungs	160
Rabbit 7	Liver	Portal vein	Lungs	0[1]
Rabbit 8	Liver	Portal vein	Lungs	120
Rabbit 9	Liver	Portal vein	Lungs	170
Rabbit 10	Liver	Portal vein	Lungs	50
Rabbit 11	Liver	Portal vein	Lungs	170
Dog 1	Spleen	Splenic artery	Liver	370
Dog 2	Spleen	Splenic artery	Splenic vein	160
Dog 3	Spleen	Splenic artery	Splenic vein	170
Dog 4	Spleen	Splenic artery	Splenic vein	170
Dog 5	Spleen	Splenic artery	Liver	200
Rabbit 12	Lungs	Right ventricle	Liver	230
Rabbit 13	Lungs	Pulmonary artery	Liver	160
Rabbit 14	Lungs	Pulmonary artery	Liver	290
Rabbit 15	Lungs	Pulmonary artery	Liver	190
Rabbit 16	Lungs	Marginal ear vein	Liver	160
Rabbit 17	Lungs	Marginal ear vein	Liver	190
Cat 1	Lungs	External jugular vein	Liver	390
Dog 6	Lungs	Pulmonary artery	Liver	100
Dog 7	Lungs	Pulmonary artery	Liver	180
Dog 8	Lungs	Pulmonary artery	Pulmonary vein	160

[1] No spheres recovered.

Spleen. Five dogs weighing 15 to 70 pounds were anesthetized with sodium pentobarbital and the splenic artery was exposed by an abdominal incision. Usually, one of the branches of the splenic artery just as it entered the spleen was taken as the injection site. However, when the size of the vessel necessitated a more distant injection, the accompanying mesentery and fat were ligated and cut away to prevent shunting of the spheres outside of the spleen. Glass spheres injected into the splenic artery were recovered from the liver and splenic vein of all five dogs (table 1). The maximum diameter of spheres recovered varied from 160 to 370 micra.

Lungs. Four rabbits weighing 5 to 10 pounds were anesthetized with ether, and three dogs weighing 15 to 40 pounds were anesthetized with sodium pentobarbital. The thoracic cavity was opened and artificial respiration was administered during the experiment. The suspension of glass spheres was injected into the right ventricle of one of the rabbits and into the pulmonary artery of the other rabbits and the three dogs. The liver and pulmonary vein were examined for trapped spheres. Spheres were recovered in the liver and pulmonary vein.

Since the thoracic exposure may have changed the hemodynamics of the lungs, injections were made into the marginal ear vein of two intact rabbits and into the external jugular vein of a cat. As before, the spheres were recovered in the liver, thus indicating passage through the lungs. The maximum size bead recovered (table 1) varied from 160 to 290 micra in the rabbits, 100 to 180 micra in the dogs and was 390 micra in the one cat.

DISCUSSION

Since glass spheres with diameters 20 to 40 times the average diameter of the lumina of capillaries rapidly passed through the liver, spleen and lung of living dogs, cats and rabbits, these spheres must have traversed the organs by means of noncapillary channels. Thus the existence of arterio-venous or noncapillary shunts in the liver, spleen and lungs has been demonstrated.

Regarding the liver, it should be noted that, since the spheres entered the liver via the portal vein and left the liver via the hepatic vein, venous-venous rather than arterio-venous shunts were demonstrated. Wakin and Mann (4), by a technique involving transillumination of the mammalian liver in situ, have shown arterio-venous anastomoses between the hepatic artery and the portal vein but have been unable to show similar shunts between the hepatic artery and the hepatic vein. While Brissaud and Sabourin (5) asserted that anastomoses are quite common between the terminal portal and hepatic veins, Mall (6) denies the existence of such channels by "injections made with granules which would pass through small arteries but not through the capillaries." Unfortunately, Mall does not give any details as to the size, shape and character of his granules; it may be that they were too large to go through the collateral shunts.

No reference to arterio-venous anastomoses in the spleen can be found in the available literature. Detailed investigations of the spleen, based upon microscopic studies of both transilluminated and fixed specimens, have been made by MacNeal (7), Robinson (8), Knisley (9), MacKenzie *et al.* (10) and Snook (11). All of these observers, however, failed to report arterio-venous shunts in this organ.

Extensive studies on the circulation of the lungs have failed to reveal the presence of arterio-venous anastomoses (12). Brenner (13), in a thorough review of the histology of the pulmonary vessels, makes no mention of such channels. Burchell and Clagett (14) have reported a case of an arterio-venous fistula in the lungs and refer to eight similar cases previously reported in the literature. Such fistulae could develop from normal arterio-venous anastomoses demonstrated in this study to be present and patent in the living organism. It is of interest in this connection that Clark and Clark (15) have observed the formation of new arterio-venous anastomoses in the ear of the adult rabbit.

In this study no exact anatomical localization of the arterio-venous shunts in the three organs was attempted. An indication of the size of the shunts is given in table 1, but it should be recognized that many of the larger spheres may have settled in vessels or organs other than those used for recovery. Therefore, it is possible that the liver, spleen and lungs may contain larger arterio-venous anastomoses than those represented by the data of these experiments. The possibility that the capillaries may have accommodated their lumina to 20 or 40 times in diameter for the short time (30 seconds or less) of these experiments appears to be remote. Since glass spheres pass through the arterio-venous anastomoses of the liver, spleen, lungs, heart (2) and kidneys (3) of living animals under normal conditions, obviously blood must also pass through these channels. Whether the blood that traverses these connections is of physiological significance is unknown. The occurrence of arterio-venous anastomoses in the organs and tissues summarized by Clara (1), together with those organs studied in this laboratory, indicates a universal occurrence of arterio-venous anastomoses in the body. It thus appears that blood can circulate through tissues by two routes: *a*) via capillaries and *b*) via arterio-venous anastomoses. The rôle played by the capillaries is well known, but whether or not the arterio-venous anastomoses perform any useful function awaits further study.

SUMMARY

1. A technique involving the injection into an afferent blood vessel and the recovery from an efferent blood vessel of glass spheres much larger than capillary size was utilized to demonstrate arterio-venous anastomoses in the liver, spleen and lungs.
2. Such glass spheres traversed the circulation of the liver of living rabbits, the spleen of living dogs and the lungs of living rabbits, cats and dogs, thus demonstrating that arterio-venous anastomoses are present and patent in these organs in vivo.
3. An indication of the size of the arterio-venous shunts is given by the diameters of the recovered spheres. The maximum diameter ranged from 50 to 180 micra in the liver, 160 to 370 micra in the spleen and 100 to 390 micra in the lungs.
4. It is suggested that there is a universal occurrence of arterio-venous anastomoses in the body.

REFERENCES

1. Clara, M. Die Arterio-Venosen Anastomosen. Johann Ambrosius Barth, Verlag, Leipzig, 1939.

2. Prinzmetal, M., B. Simkin, H. C. Bergman and H. E. Kruger. Am. Heart J. **33:** 420, 1947.
3. Simkin, B., H. C. Bergman, H. Silver and M. Prinzmetal. Arch. Int. Med. In press.
4. Wakin, K. G. and F. C. Mann. Anat. Rec. **82:** 233, 1942.
5. Brissaud and Sabourin. Compt. rend. Soc. de biol. **40:** 757, 1888.
6. Mall, F. P. Am. J. Anat. **5:** 227, 1906.
7. MacNeal, W. J. Arch. Path. **7:** 215, 1929.
8. Robinson, W. L. Am. J. Path. **6:** 19, 1930.
9. MacKenzie, D. W., Jr., A. O. Whipple and M. P. Wintersteiner. Am. J. Anat. **68:** 397, 1941.
10. Knisley, M. H. Anat. Rec. **65:** 23, 1936.
11. Snook, T. Anat. Rec. **89:** 413, 1944.
12. Miller, Wm. Snow. The Lungs. Springfield, Ill.: Charles C Thomas, 1937.
13. Brenner, O. Arch. Int. Med. **56:** 211, 1935.
14. Burchell, M. B. and O. T. Clagett. Am. Heart J. **34:** 151, 1947.
15. Clark, E. R. and E. L. Clark. Am. J. Anat. **55:** 507, 1934.

SOME ACTIONS OF POTASSIUM CHLORIDE AT THE NEUROMUSCULAR JUNCTION

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It is said that potassium plays an important part in the chemical transmission of the nerve impulse. Several studies have been made of its effect on muscular contraction produced by indirect stimulation at low frequency (1–4). Furthermore, it has been demonstrated that eserine potentiates the effect of potassium chloride in the superior cervical ganglion of the cat (5), and that potassium in turn augments the action of acetylcholine in the ganglion (5, 6) and in the anterior rectus of the toad (7–9). The objectives of the present study were to study the action of potassium on normal and denervated muscles stimulated electrically or by means of acetylcholine; to determine whether or not mammalian muscle responds to potassium in the same way as amphibian muscle or the superior cervical ganglion; and to attempt to determine its mechanism of action by comparing its effects with those of acetylcholine.

METHODS

Cats were used, anesthetized with 1 cc. per kgm. of a 25 per cent solution of urethane (Merck) which contained 3.3 per cent of sodium pentobarbital (Nembutal, Abbott). The muscles employed were the quadriceps and the soleus, the femur or the tibia being fixed by means of drills as necessary. In preparing denervated muscles, the sciatic was sectioned aseptically 6 to 14 days previously. To stimulate the muscles either directly or indirectly through the nerve, we used condenser discharges or induction shocks from a Harvard inductorium with the primary connected to a source of alternating current of 50 cycles per second at three volts, and the secondary set to produce maximal stimuli. For indirect stimulation silver electrodes insulated with rubber were used, and for direct stimulation steel hooks, one of which was placed in the corresponding tendon and the other in the muscle mass. Stimuli were always maximal. The muscular contractions were recorded on a kymograph by fastening the corresponding tendon to the short end of a lever loaded with rubber bands.

The drugs used were acetylcholine (Roche), prostigmine (Roche), potassium chloride (Merck), atropine sulfate (Mallinckrodt) and curare (Merck). All were dissolved in distilled water and were injected rapidly into the abdominal aorta below the inferior mesenteric artery, with the exception of the atropine which was given by vein. Acetylcholine (a.c.) was always injected in a volume of 0.10 cc.; potassium chloride (KCl) was employed in 5 per cent solution when up to 10 mgm. were injected in all, and in 20 per cent solution when a larger amount was injected. In the section devoted to results and in the figures the quantities of KCl in mgm. used for each leg of a cat in a given experiment are indicated. When corresponding muscles of both legs were employed the medial sacral artery was ligated; when a muscle of one side only was used, this artery was ligated and also the contralateral common iliac.

RESULTS

1. *Action of KCl on denervated muscle.* Potassium chloride may produce contraction or contracture of denervated muscle whether mammalian or amphibian (2, 10, 12–15). Acetylcholine produces a similar effect. It has been found that the minimal dose of KCl capable of producing shortening of the soleus muscle denervated 6 to 14 days previously is the same as that necessary to cause a contraction[1] in the normal soleus. This is always the case when both muscles are equally loaded and the same amplification is maintained in the recording. The contradictory results reported by various authors (8, 12, 14) may be due to the fact that determinations of the minimal dose of KCl required to produce muscular contraction were made without taking into consideration the loads on the two muscles. In this respect there is a great discrepancy between KCl and a.c., since denervated muscle becomes highly sensitized to the action of a.c., a fact which has been known for a long time.

2. *Action of KCl during indirect stimulation.* The effects of potassium chloride depend upon the frequency of stimulation and the quantity injected. With stimuli at a frequency of between 0.1 and 9 per second, 5 mgm. of potassium chloride produced in all the animals studied a transitory potentiation of the muscle twitches (fig. 1a), a phenomenon which has already been observed by Brown and von Euler (3). (In this work a twitch is considered to be potentiated as compared with another only when the contraction reaches a greater height above the previous base line, maximal stimuli being used throughout.) In 14 experiments out of 17, 10 mgm. of KCl caused a further increase in tension (fig. 1a); in the remaining experiments this greater development of tension was preceded by a depressive phase (fig. 3a and 5a), a phenomenon which has been described by Brown (2). With doses of 20 to 40 mgm. it is observed that the contraction produced by KCl has superimposed upon it the twitches produced by indirect stimulation. The height of the twitches superimposed upon the contraction produced by KCl is less than the height of those which occurred before the injection of this substance. When the contraction in response to potassium has disappeared, a depressive phase occurs, followed by a potentiation of the twitches (fig. 1a). If the dose is greater, or if small doses are injected repeatedly in a short period of time, the contraction produced by KCl is followed by a long depression of the twitches (fig. 3b, 3); in some instances after the depression a

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[1] For the sake of convenience of expression, the term *contraction* will be used to designate any shortening of the muscle without distinction between contraction and contracture.

NOTE. While this paper was being translated into English by the Committee on Inter-American Scientific Publication (Cambridge, Massachusetts), we noticed the S. M. Walker's reports (for references see: Proc. Soc. Exp. Biol. and Med. 64: 425, 1947) dealing with this subject. The conclusions are similar to our work.

potentiation of the contractions occurs (fig. 3a). Various authors who have used conditions similar to ours have obtained with a.c. only depressor effects similar to those described here for KCl (18–21). Only Simonart (22) has observed transient potentiation produced by a.c., which apparently was due to vascular changes (20).

During incomplete tetanus KCl has various actions which depend upon the tension produced by the muscle and the amplitude of the twitches. If little tension is developed and the twitches are large, KCl produces the effect presented in figure 1b, with only quantitative variations from one animal to another.

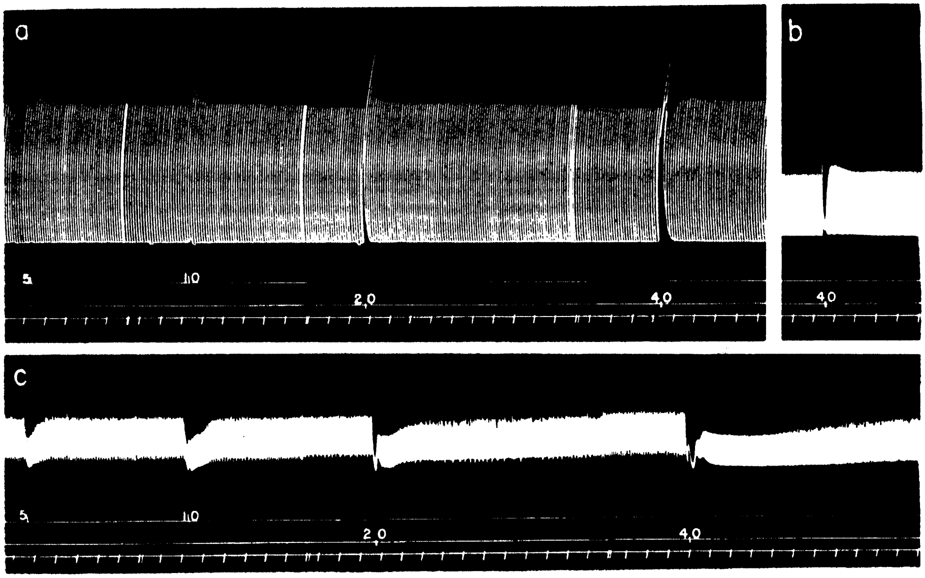


Fig. 1. Effect of KCl on contraction produced by indirect stimulation. Cat anesthetized with nembutal. Quadriceps muscle. All signals indicate injections of KCl, with the doses expressed in milligrams. (See *Methods*.) Time in minutes. Frequency of stimulation: *a*) 48 per minute; *b*) 7 per second; *c*) 13 per second.

When the muscle develops more tension and the amplitude of the twitches is less, KCl causes depression with modifications of tension and amplitude which are more complex with larger doses (fig. 1c). After amounts greater than 40 mgm. one observes only a contraction followed by depression which lasts 10 minutes or more.

The action of KCl in muscles stimulated at frequencies which give complete tetanus depends not only on the dose but also upon the stage of synaptic transmission during which the substance is injected. In the first stage small amounts (5 to 10 mgm.) produce a slight increase in tension proportional to the dose injected (fig. 2a); larger amounts (20 to 40 mgm.) reduce the tension in proportion to the dose (fig. 2a). In a muscle in the third stage, KCl produces a decrease of tension developed by the muscle, larger doses producing greater depression;

later a potentiation occurs which is inversely proportional to the amount of KCl injected (fig. 2c). In the fourth stage 5 to 40 mgm. of KCl causes a rapid increase of tension followed by a decline; after the depression a new increase in tension occurs. In general these changes are proportional to the doses employed (fig. 2b). If the quantity of KCl injected is excessive (60 mgm.), a small notch appears on the descending limb of the depression (fig. 2a, 2c and 2d).

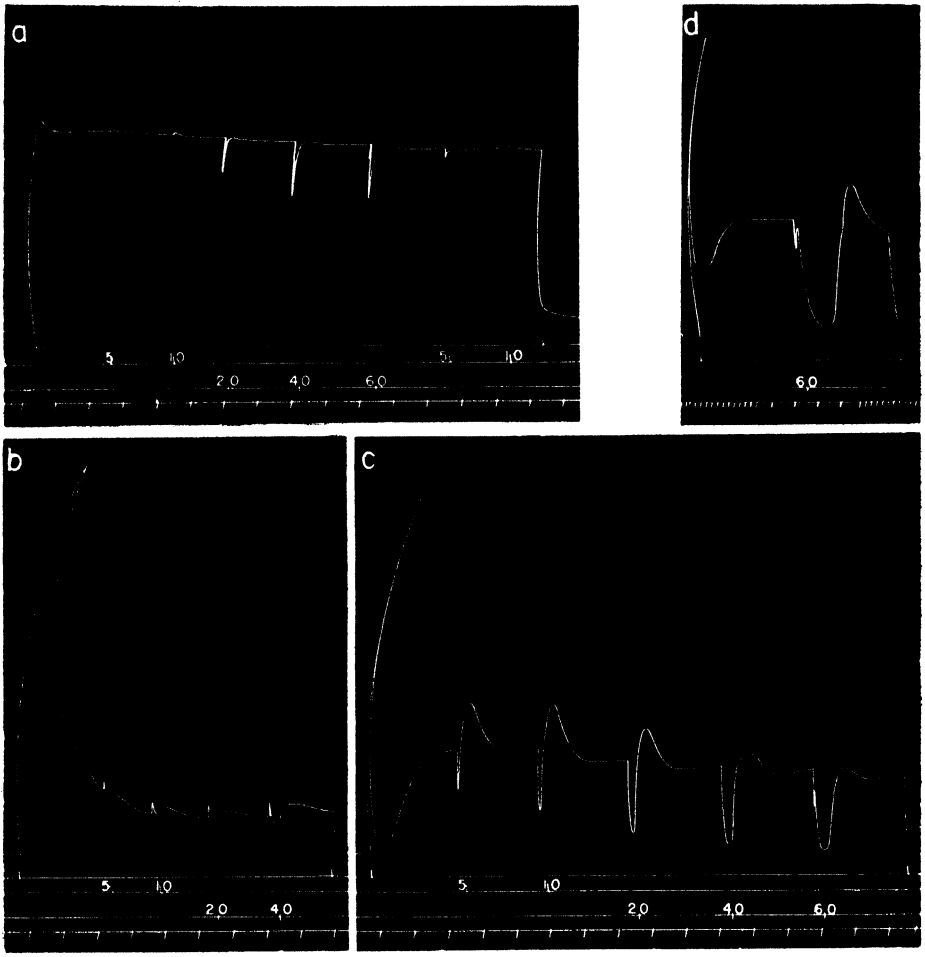


FIG. 2. EFFECT OF KCl ON CONTRACTION produced by indirect stimulation. Cat anesthetized with nembutal. Quadriceps muscle. Upper signal line indicates the beginning and end of stimulation; middle and lower lines indicate injections of KCl; doses are expressed in milligrams. Frequency of stimulation: *a*) 19 per second, time in minutes; *b*) 50 per second, time in minutes; *c*) 300 per second, time in minutes; *d*) 300 per second, time 10 seconds.

Acetylcholine causes effects which are closely similar to those described for KCl in muscles contracted tetanically by indirect stimulation (18, 23).

3. *Action of KCl during direct stimulation.* Just as in the case of indirect

stimulation of muscle, the effects of KCl depend upon the frequency of stimulation and the amount injected.

With frequencies between 0.2 and 9 per second, 2.5 mgm. of KCl produced in 13 animals a further increase in tension, and, furthermore, in three cases a small contraction was observed (fig. 3a). At the same frequency, doses of 10 to 30 mgm. caused in 12 experiments an increase in the tension of the muscle twitches which in 8 cases is preceded by the contraction produced by KCl. With still larger amounts there is always muscular contraction followed by a depression (fig. 3b); recovery requires 10 minutes or more.

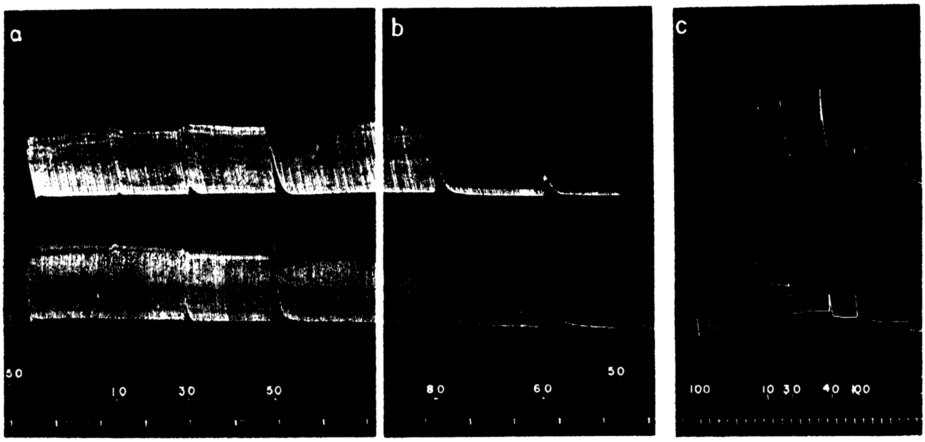


FIG. 3. EFFECT OF KCl ON CONTRACTION produced by indirect stimulation (*upper record*) and by direct stimulation (*lower record*). Soleus muscle. (The muscle stimulated directly was denervated 14 days previously.) Cat anesthetized with nembutal. Time in minutes. *a*) Frequency of stimulation: 72 per minute. *Upper line:* injection of 50 micrograms a.c.; *middle line:* injections of KCl, doses in milligrams. *b*) Three minutes after *a*, with same frequency of stimulation. *Upper line:* injection of 50 micrograms a.c.; *middle line:* injections of KCl, doses in milligrams. *c*) Thirty minutes after *b*; frequency of stimulation: 40 per second. *Upper line:* beginning and end of stimulation; *middle line:* first and last signals: 100 micrograms a.c.; other signals indicate injections of KCl, doses in milligrams.

In the case of muscles in incomplete tetanus, 2.5 to 10 mgm. produces changes in tension which vary from one experiment to another. Either an increase or a decrease in the amplitude of the twitches may be observed, while in other cases a definite contraction is produced. In general, the depressive effects predominate, whether or not contraction occurs. Larger doses (20 mgm. or more) produce a contraction which is sometimes preceded by a slight depression. In other cases there is no contraction, but there is always a terminal depressive phase which may last 10 minutes or more. In the case of muscles in complete tetanus with the tension diminishing, the action of KCl depends upon the dose injected: small amounts cause a depression with a tendency for recovery to occur and for the tension to exceed the original, larger doses always produce a contraction followed by a long depression which lasts 10 minutes or more (fig. 3c).

The inhibition produced by KCl in denervated muscles stimulated electrically has been observed by Gasser and Dale (24). During the depression, provided the dose of KCl is not excessive, the response to an injection of a.c. is not significantly altered (fig. 3c). In denervated muscles a.c. can produce the same effect. Rosenblueth and Luco (17) interpret this by assuming that a.c. depolarizes the membranes of the muscle fibres, which would interfere with direct electrical stimulation but would not alter stimulation by means of a.c. A similar hypothesis is acceptable in the case of KCl, since this substance is able to depolarize the muscle membrane (3, 12).

4. *Action of KCl after prostigmine.* All of the experiments included in this section were carried out on the quadriceps.

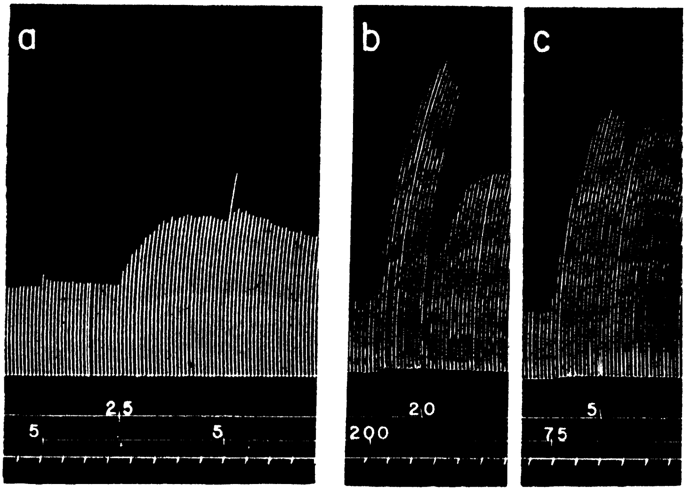


FIG. 4. EFFECT OF KCl ON POTENTIATION by prostigmine. Cat anesthetized with nembutal. Quadriceps muscle, stimulated indirectly once per 10 sec. Time in minutes. *a) Upper line:* injection of 25 micrograms prostigmine; *middle line:* injections of KCl, doses in milligrams. *b) Upper line:* injection of KCl, dose in milligrams; *middle line:* injection of prostigmine, dose in micrograms. *c)* Same animal when height of twitches was equal to that at the beginning of *b*. *Upper line:* injection of KCl, dose in milligrams; *middle line:* injection of prostigmine, dose in micrograms.

The action of KCl depends upon the frequency of stimulation and on the doses of prostigmine and of KCl used. With stimuli at very low frequencies (1 per 10 sec.), 25 micrograms of prostigmine causes a slight potentiation of the twitches (fig. 4a). If 5 mgm. of KCl is injected during this potentiation, the increased tension developed in the twitches is greater than that produced by the same dose of KCl before the injection of prostigmine (fig. 4a). This phenomenon has not been observed in the case of a.c. by investigators using conditions similar to ours (20). If the dose of prostigmine is increased so as to cause a large potentiation of the twitches, 5 mgm. of KCl causes a slight depression of these (fig. 4c), while larger amounts reduce them considerably (40 mgm., fig. 4b). A similar effect is obtained with a.c. injected under these conditions (18, 20).

With larger doses of prostigmine (250 μg./kgm.) a slight potentiation of short duration is produced, followed by a depression which becomes greater if KCl is injected at this moment (fig. 5b). The larger the dose of KCl the greater is the depression produced (fig. 5b). This has also been described for a.c. (18, 20).

If the muscle is stimulated indirectly with frequencies of 1.6 to 9 per second and KCl is injected after the twitches are depressed by large doses of prostigmine,

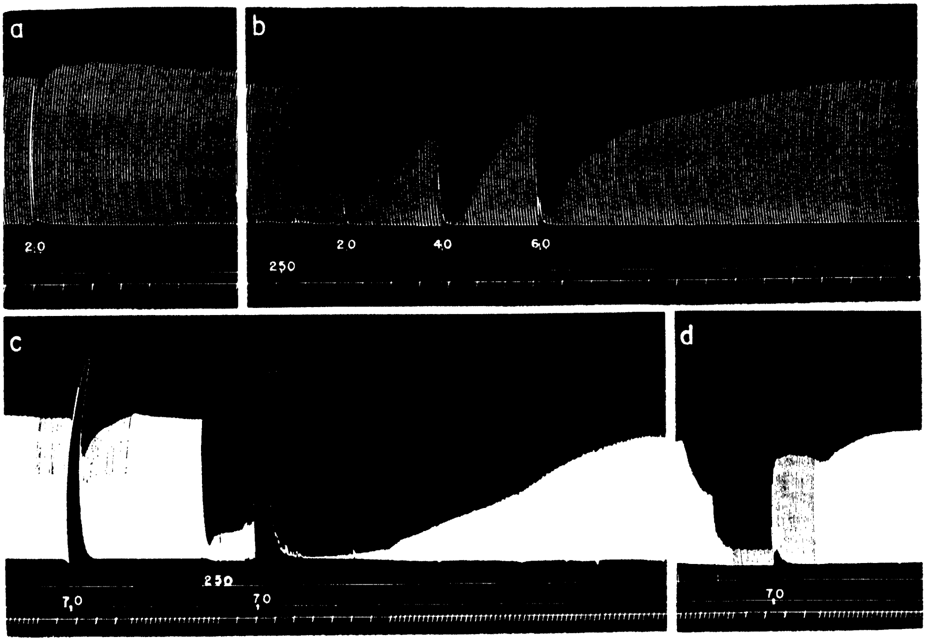


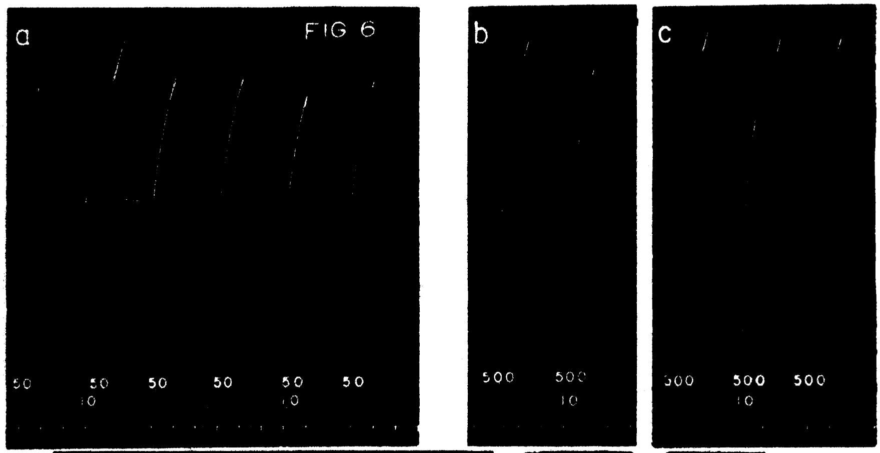
FIG. 5. SYNERGISTIC ACTION OF KCl WITH PROSTIGMINE; antagonistic action to curare. Cat anesthetized with nembutal; artificial respiration; atropine, 1 mgm. per kgm. intravenously. Quadriceps muscle stimulated indirectly. *a*) Frequency of stimulation, 10 per minute. *Upper line:* injection of KCl, dose in milligrams. Time in minutes. *b*) Three minutes after *a*, same frequency of stimulation. *Upper line:* injections of KCl, doses in milligrams; *middle line:* injection of prostigmine, 250 micrograms per kgm. Time in minutes. *c*) Frequency of stimulation, 100 per minute. *Upper line:* injection of prostigmine, dose in micrograms; *middle line:* injections of KCl, doses in milligrams. Time: 10 seconds. Variations in spacing of time signals are due to changes in speed of kymograph. *d*) Fifteen minutes after *c*, same frequency of stimulation. *Upper line:* injections of curare; *middle line:* injection of KCl, dose in milligrams. Time as in *c*.

three phenomena occur: the twitches disappear during the contraction produced by KCl, the muscular fibrillation which is characteristic of the action of prostigmine is intensified and the contractions caused by electrical stimulation are further depressed (fig. 5c).

5. *Action of KCl after curare.* KCl has a decurarizing action (fig. 5d), which has already been described by other authors (3, 10). The same phenomenon has been described for a.c. (10, 19, 25).

6. *Action of KCl on muscular contraction produced by intra-arterial injection*

of a.c. When the denervated soleus is stimulated periodically with small amounts of a.c. (20 to 60 μg.), injections of KCl have different effects, depending upon the time interval which separates the injections of the two drugs. If KCl is injected 5 to 15 sec. before the next dose of a.c., the contractions produced by the latter drug are smaller or equal to that produced previously (fig. 6a). When longer intervals of time are used (15 to 30 sec.) the response to a.c. is potentiated (fig. 6a). This potentiation has been described also in the anterior rectus of the road (7–9). On the other hand, Ravin (26) has observed no increase in the



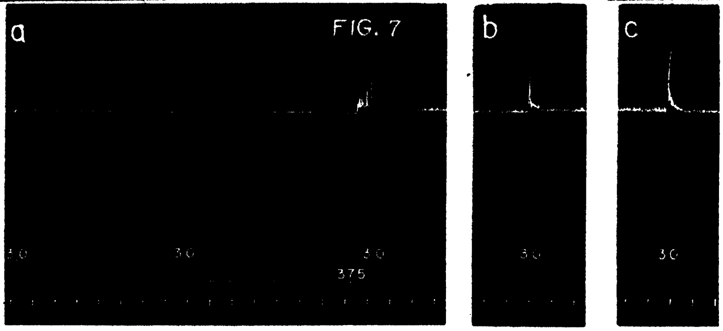
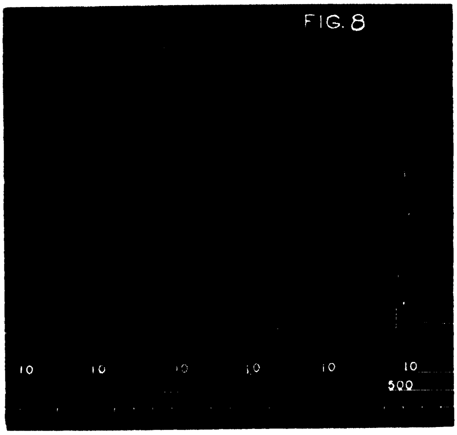


FIG. 6. EFFECT OF KCl ON CONTRACTION produced by a.c. Cat anesthetized with nembutal. *Upper record,* denervated soleus, *lower record,* normal soleus. *Upper lines:* injections of a.c., doses in micrograms; *middle lines:* injections of KCl, doses in milligrams. Time in minutes. *a*) Soleus denervated 11 days previously. The first injection of KCl was given 20 seconds before the injection of a.c. The second injection of KCl, 8 seconds before. *b*) Soleus denervated 8 days previously. The injection of KCl was given 15 seconds before the injection of a.c. *c*) Same animal as in *b*, 10 minutes later. Procedure as in *b*, but injection of KCl given 25 seconds before a.c.

FIG. 7. ACTION OF PROSTIGMINE ON CONTRACTION produced by KCl. *Upper record,* soleus denervated 7 days previously; *lower record,* normal soleus. Cat anesthetized with nembutal; atropine, 1 mgm. per kgm., intravenously. Time in minutes. *a*) *Upper line:* injections of KCl, doses in milligrams; *middle line:* injection of 375 micrograms prostigmine. *b*) Sixteen minutes after *a*. *c*) Thirty-two minutes after *a*.

responses to a.c. after KCl in the gastrocnemius of the cat. His results may be due among other tings to the fact that the injections of a.c. were separated by only a very short time (15 sec.) from those of KCl, or perhaps to the fact that the gastrocnemius behaves in this respect in a different way from the soleus and the anterior rectus of the toad.

If the amount of a.c. is sufficient to produce a maximal response, the KCl injected immediately before reduces the height of the contraction (fig. 6b); if the interval between the two injections is increased, the contraction is at most of the same height as the previous contraction, but never greater (fig. 6c).



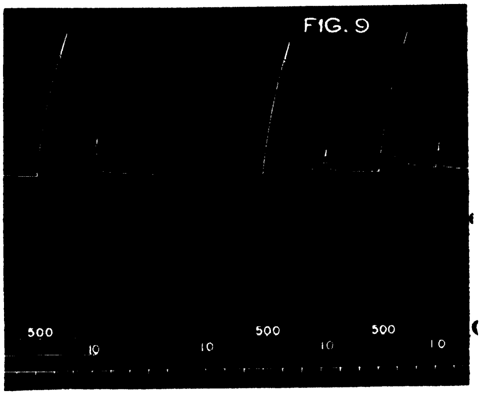


FIG. 8. EFFECT OF TETANIC CONTRACTION upon the response of the muscle to an injection of KCl. *Upper record,* normal soleus; *lower record,* soleus denervated 7 days previously. Cat anesthetized with nembutal. Time in minutes. *Upper line:* injections of KCl, doses in milligrams; *middle line:* first two signals, beginning and end of stimulation (indirect *above,* direct *below*); third signal, injection of 500 micrograms a.c. (Both the tetanus and the injection of a.c. occurred 20 seconds before the injection of KCl.)

FIG. 9. EFFECT OF CONTRACTION produced by a.c. on response of muscle to KCl. *Upper record,* soleus denervated 9 days previously; *lower record,* normal soleus. Cat anesthetized with nembutal. Time in minutes. *Upper line:* injections of a.c., doses in micrograms; *middle line:* injections of KCl, doses in milligrams.

In the normal muscle, it is also possible to observe potentiation of the responses to a.c. after an injection of KCl, provided the interval between the two injections is greater than 10 seconds (fig. 6b and 6c).

7. *Action of prostigmine on muscular contractions produced by KCl.* When muscular contractions are produced by means of injections of KCl, large doses of prostigmine potentiate the response of normal muscle, but cause no change in denervated muscle (fig. 7a, b, c). On the other hand, prostigmine potentiates the action of a.c. both in normal and in denervated muscle (16).

8. *Post-tetanic sensitization to KCl.* A tetanus of 15 sec. duration produced by indirect stimulation of the normal soleus potentiates the response to an injection of KCl made immediately afterward (fig. 8). This fact has been described previously by Northrop and Gellhorn (27). In the denervated soleus a tetanus

of equal duration produced by direct stimulation does not cause any change in the contraction produced by KCl (fig. 8), while the responses to injections of a.c. are potentiated (17).

9. *Sensitization to KCl produced by a.c.* A sufficient dose of a.c. potentiates the contraction produced by a subsequent dose of KCl, a phenomenon which is seen in both normal and denervated muscle (fig. 8 and 9). This has been observed in the superior cervical ganglion (5). This sensitization appears at least three minutes after the injection of a.c. (fig. 9) and disappears after a time. If periodic contractions are produced by means of a.c. with interpolated injections of KCl, this sensitization lasts for a long time (fig. 9). Sensitization to KCl by indirect stimulation has these same characteristics.

DISCUSSION

It may be accepted as a general hypothesis that one or more processes intervene between the stimulus and the specific response of the effector. This supposition seems to hold true both in the striated muscles of the mammal and the amphibian and in the superior cervical ganglion. Apparently these processes are qualitatively the same in the three effectors because they are known to react in much the same way to many drugs and types of stimulation. If KCl controls one or more processes which are qualitatively the same in amphibian and mammalian muscle and in the superior cervical ganglion, it must have a similar action in all of the three effectors. This is, in fact, the case. The similarity of the action of KCl on these structures can be deduced from observations published by other authors and from the results of this investigation. In fact, KCl can stimulate all the effectors mentioned (2, 5, 6, 8, 10, 12–15 and section 1); in all of them it can potentiate the stimulating action of a.c. (5, 7–9 and section 6) or the contractions produced by indirect stimuli (1, 3, 5, 6, 28 and section 2). In the same way it can inhibit the responses of these structures when stimulated indirectly or by a.c. (2, 3, 5, 6, 28, 29 and sections 2 and 6). The fact that eserine potentiates the response of the superior cervical ganglion to KCl (5) and prostigmine potentiates the response of mammalian muscle gives further support to this proposition.

Furthermore, potassium apparently plays an important part in the processes occurring between the stimulus and the response of striated muscle. A series of well-known facts supports this supposition: potassium causes muscular contraction; whenever contractions occur, potassium is liberated; there are many close similarities between the actions of this substance and the nicotine-like effects of a.c. (for general references see 30); potassium can reinforce the effect of a.c., and a.c. in turn can reinforce the effect of potassium (sections 6 and 9), etc. In view of the similarities between the actions of potassium and of a.c., and in view of the fact that these actions potentiate each other, it might be supposed that a.c. stimulates muscle through a chain of processes one of which involves potassium. The latter substance must act at a step later than a.c., since curare blocks the contractions produced by a.c. but not those produced by potassium (2). The same selective block is produced in the anterior rectus of the toad by curare, erythroidine, cobra venom, strychnine and atropine (8).

There are, none the less, differences between the effects produced by a.c. and by potassium: *a*) the effects of potassium are more prolonged than those of a.c.; *b*) the injection of potassium during indirect stimulation at low frequency produces potentiation of the twitches (section 2), a phenomenon which has not been observed with a.c. even by authors who have worked in conditions similar to ours (18–20); *c*) in striated muscles stimulated indirectly at low frequency, potassium can increase the size of the twitches after prostigmine (section 4), which has also not been described for a.c. (18–20); *d*) denervated muscle does not become sensitized to the action of potassium (section 1); and *e*) prostigmine potentiates the contraction produced by potassium in normal muscle, but does not do so in denervated muscle (section 7). On the other hand this drug potentiates the action of a.c. in both cases (16); tetanic stimulation of a denervated soleus causes potentiation of the responses to a.c. and not of those produced by potassium (17 and section 8).

Other differences between the effects of potassium and of a.c. in striated mammalian and amphibian muscle have been described. Some have already been analyzed in this discussion (2, 8); the others do not modify the general plan of the problem in any way, for which reason they will not be considered (12, 31).

The differences described in *a*, *b* and *c* may be explained by the fact that potassium disappears much more slowly than a.c., for which reason its effects can be more prolonged. The other differences have a more complex origin; however, in order to explain them it does not seem necessary to postulate that KCl stimulates the muscle through a chain of processes different from those involving a.c. Thus the fact that a tetanus is able to augment the effect of a subsequent injection of KCl in normal muscle may be due to the fact that during indirect stimulation of the muscle a.c. is liberated, which augments the effects of KCl as has been shown (section 9). On the other hand the well-known post-tetanic sensitization of normal or denervated muscle to a.c. is thought to be due to quite different phenomena (3, 17, 18).

In order to explain the lack of sensitization of denervated striated muscle to the action of KCl (section 1) it may be suggested that this element, as has already been discussed above, acts upon a later step than does a.c. Denervation may sensitize only the first processes which mediate between the stimulus and the response of the effector.

Prostigmine also potentiates the contraction produced by KCl only in normal muscle. This may be due to the liberation of a.c. at the motor end-plates, since Masland and Wigton (32) observed spontaneous discharges in motor nerve fibres after prostigmine. The same phenomenon has been described by Eccles, Katz and Kuffler (33) after eserine. On the other hand, prostigmine may potentiate the effects of a.c. either by inhibiting cholinesterase or by acting directly upon the muscle fibre (34, 35) to sensitize the first processes which mediate between the stimulus and the response.

Some of the actions of KCl may be due to the inhibition of cholinesterase caused by this substance. Mendel, Mundell and Strelitz (36) obtained inhibition of the enzyme in vitro up to 40 per cent with 20 per cent solutions of KCl. The previous mechanism, however, does not explain all of the actions of KCl. KCl causes changes in denervated muscles (sections 1, 3, 6, 7–9), a fact which is impossible to explain on this basis. Furthermore, its action occurs for several minutes in the leg muscles of the cat after small doses are injected into the abdominal aorta without ligation of any artery except the medial sacral (fig. 3a and 5a). Using such quantities of KCl, it appears to be very difficult to obtain lasting concentrations of KCl at the motor end-plate whose value approximates that used by Mendel, Mundell and Strelitz. Furthermore these authors (36) have observed that small concentrations of calcium or magnesium ions such as occur normally in the blood interfere with the anti-cholinesterase action of KCl. Consequently, if inhibition of cholinesterase plays any part in the action of KCl, it must be slight.

SUMMARY

1. The action of KCl injected into the terminal portion of the abdominal aorta has been studied in the normal quadriceps and in the normal and denervated soleus of the cat.

2. KCl causes contraction of the soleus which has been denervated 6 to 14 days previously. The minimum dose capable of producing this effect is equal to the minimum quantity required to produce a contraction of the normal control soleus (section 1).

3. In the quadriceps or soleus stimulated indirectly KCl can produce contraction, potentiation or depression of the response of the muscle depending upon the frequency of stimulation and the dose employed (section 2).

4. In the soleus denervated 6 to 14 days previously and stimulated directly by electric shocks, KCl produces the same effects as in the normal soleus stimulated indirectly (section 3).

5. In the quadriceps stimulated indirectly at low frequency KCl may have effects which are synergistic or antagonistic to those produced by a previous injection of prostigmine; the type of effect will depend upon the frequency of stimulation and the dose of prostigmine and KCl used (section 4).

6. KCl has a decurarizing action (section 5).

7. In both the denervated and the normal soleus KCl can produce either depression or potentiation of the contractions evoked by injections of a.c., depending upon the time interval between the two injections (section 6).

8. In the normal soleus prostigmine potentiates the contraction produced by KCl, but this effect is not observed in the soleus denervated 6 to 14 days previously (section 7).

9. A tetanus produced by indirect stimulation potentiates the response of the normal soleus to KCl (section 8).

10. A.c. sensitizes the normal or denervated soleus to a subsequent injection of KCl (section 9).

11. The effects of KCl are discussed in relation to the mechanism of action of a.c. at the neuromuscular junction.

REFERENCES

1. Grumbach, L. and D. T. Wilber. This Journal **130**: 433, 1940.
2. Brown, G. L. J. Physiol. **91**: 4P, 1937.

3. Brown, G. L. and U. S. von Euler. J. Physiol. **93:** 39, 1938.
4. Baetjer, A. M. This Journal **112:** 147, 1935.
5. Feldberg, W. and A. Vartiainen. J. Physiol. **83:** 103, 1934.
6. Brown, G. L. and W. Feldberg. J. Physiol. **84:** 12P, 1935.
7. Wacholder, K. and F. Matthias. Pflüg. Arch. ges. Physiol. **232:** 159, 1933.
8. Cicardo, V. H. Rev. Soc. Arg. Biol. **15:** 12, 1939.
9. Altamirano, M. Unpublished work.
10. Wilson, A. T. and S. Wright. Quart. J. Exp. Physiol. **26:** 127, 1937.
11. Brown, G. L. and W. Feldberg. J. Physiol. **86:** 10P, 1936.
12. Kuffler, S. W. J. Neurophysiol. **6:** 99, 1943.
13. Buchtal, F. and J. Lindhard. J. Physiol. **95:** 59P, 1939.
14. Solandt, D. Y. and J. W. Magladery. J. Neurophysiol. **5:** 357, 1942.
15. Dale, H. H. and H. S. Gasser. J. Pharmacol. and Exper. Therap. **29:** 53, 1926. Cited by Lyman Ch. P. This Journal **137:** 392, 1942.
16. Brown, G. L. J. Physiol. **89:** 438, 1937.
17. Rosenblueth, A. and J. V. Luco. This Journal **120:** 781, 1937.
18. Rosenblueth, A. and R. S. Morison. This Journal **119:** 236, 1937.
19. Rosenblueth, A., D. B. Lindsley and R. S. Morison. This Journal **115:** 53, 1936.
20. Brown, G. L., H. H. Dale and W. Feldberg. J. Physiol. **87:** 394, 1936.
21. Bacq, Z. M. and G. L. Brown. J. Physiol. **89:** 45, 1937.
22. Simonart, A. Arch. int. Pharmacodyn. **51:** 381, 1935. Cited by 20.
23. Rosenblueth, A. and W. B. Cannon. This Journal **130:** 205, 1940.
24. Gasser, H. S. and H. H. Dale. J. Pharmacol. **28:** 287, 1926. Cited by 16.
25. Briscoe, G. J. Physiol. **87:** 425, 1936.
26. Ravin, A. This Journal **131:** 216, 1940.
27. Gellhorn, E. and D. Northrop. This Journal **100:** 173, 1932.
28. Brown, G. L. and W. Feldberg. J. Physiol. **86:** 290, 1936.
29. Cicardo, V. H. Rev. Soc. Arg. Biol. **14:** 370, 1938.
30. Fenn, W. O. Physiol. Rev. **20:** 377, 1940.
31. Brown, G. L. and F. C. MacIntosh. J. Physiol. **96:** 10P, 1939.
32. Masland, R. L. and R. Wigton. J. Neurophysiol. **3:** 269, 1940.
33. Eccles, J. C., B. Katz and S. W. Kuffler. J. Neurophysiol. **5:** 211, 1942.
34. Riker, W. F. and W. Clarke Wescoe. J. Pharmacol. and Exper. Therap. **88:** 58, 1946.
35. Miquel, O. J. Pharmacol. and Exper. Therap. **88:** 67, 1946.
36. Mendel, B., D. Mundell and F. Strelitz. Nature **144:** 479, 1939.

EXCHANGE AND DISTRIBUTION OF FLUID IN DEHYDRATION IN THE DOG

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In the original studies of dehydration by Gamble and his associates (1) and in several subsequent investigations (2–4) the fluid changes were calculated from the electrolyte balance and from the alterations in concentration of serum proteins and the hematocrit values. The dilution methods for measuring plasma volume, extracellular fluid volume and total body water offer another avenue of approach to this problem. Observations with these techniques formed the basis of our preliminary report (5) and a similar approach has been used more recently by others (6, 7). The scope of our earlier investigation (5) was subsequently extended to include simultaneous electrolyte balance studies. This was done to determine the relation of the electrolyte balance to the volume changes in the three fluid compartments. These combined studies form the basis of this report.

Two methods of dehydration were chosen for investigation. In the first series of experiments dehydration was produced by deprivation of water and food. This form of dehydration is, of course, complicated by nutritional disturbances, accentuating the loss of nitrogen and potassium (1) and exaggerating the loss of intracellular fluid. Another feature of this type of gradually developing dehydration is that the plasma protein concentration gives no indication of the extent of the loss in plasma volume. In order to eliminate the complications associated with malnutrition, another series of experiments was performed in which rapid water loss was produced by the intravenous injection of hypertonic sucrose solution. The entire cycle could then be completed in 24 hours with a minimal urinary loss of potassium and nitrogen. The two forms of dehydration were compared with respect to *a*) electrolyte balance, *b*) plasma volume changes and *c*) the extent to which each fluid compartment adjusts to water loss.

MATERIALS AND METHODS

Fifteen mongrel dogs weighing 10 to 15 kgm. were used for the experiments. Plasma volume and available fluid were determined by the procedure of Gregersen and Stewart (8) and the total body water was measured by the method of Painter (9). The serum protein concentration was determined in all experiments from the refractive index of the serum (10). Confirmatory data were obtained in many instances by the falling drop method (11), by the Tiselius electrophoresis method and by Micro-Kjeldahl determinations. The hematocrit value was determined by centrifugation at 3000 r.p.m. for 30 minutes using heparinized blood in Wintrobe tubes. The percentage of water in blood and serum was obtained by drying the samples at 78°C. to constant weight. Blood and urine samples were dry ashed in a muffle furnace and then analyzed for sodium according to the method of Butler and Tuthill (12) and for potassium by the method of Shohl and Bennett (13). Serum chlorides were determined by the adsorption-indicator method of Saifer and Kornblum (14) and frequently checked by the technique of Van Slyke (15). Urine chlorides were analyzed by the Volhard titration method (29). In four experiments total urinary nitrogen was determined by the Micro-Kjeldahl method.

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TABLE 1. TYPICAL EXPERIMENTS ON DOG J[1] (SPLENECTOMIZED) SHOWING THE QUANTITATIVE CHANGES IN THE FLUID COMPARTMENTS IN THE TWO TYPES OF DEHYDRATION

DATE	CONDITION	WEIGHT		TOTAL BODY WATER (T.W.)		AVAILABLE FLUID (A.F.)				INTRACELLULAR FLUID			PLASMA VOLUME (T-1824)		PLASMA PROTEIN		HEMATOCRIT	
											% change calc. from							
		Kgm.	% change	Liters	% change	Cc.	% change SCN	Calc. Cl	From Na	T.W.-A.F. cc.	T.W.-A.F.	Total base	Cc.	% change	Grams %	Calc. change P.V. %	% cells	Calc. change P.V. %
Dehydration—i.v. injection of 20 cc/kgm. 50% sucrose																		
7-10-39	Control	7.98		5.00						2210					7.7		27.7	
7-11-39	Dehydrated	7.16	10.3	4.25	15.0			24.7	16.5	2150	2.7	13.5			10.3	24.8	28.7	3.6
7-24-39	Control	8.31				2790							498		6.7		26.0	
7-25-39	Dehydrated	7.44	10.5			2100	24.7	17.9	17.9			12.2	359	27.9	8.7	23.6	25.0	4.0
Dehydration—food and water deprivation for 5 days																		
10-30-41	Control	9.89				2460							417		7.0		34.8	
11- 4-41	Dehydrated	8.64	12.7			2240	8.9						353	15.3	7.1	1.4	34.8	0
12-11-41	Control	9.15		5.10						2640			408		6.9		37.9	
12-16-41	Dehydrated	8.09	11.6	4.82	5.5			9.9	8.95	2580	2.3	7.7	335	17.9	6.7	2.5	40.4	6.2
Dehydration—food and water deprivation for 11 days																		
10-30-41	Control	9.89				2460							417		7.0		34.8	
11-10-41	Dehydrated	7.70	22.2			2020	21.9	15.0	16.6			23.1	306	29.0	6.8	2.9	33.9	2.7
12-11-41	Control	9.15		5.10						2640			408		6.9		37.9	
12-22-41	Dehydrated	7.17	21.6	4.10	19.6			17.5	15.8	2080	21.2	23.7	294	28.0	6.5	6.2	37.5	1.1

[1] Electrolyte data for corresponding experiments on this animal are presented in table 2.

During the course of the experiments, we found that a high concentration of sodium thiocyanate (above 60 mgm. per cent) in the serum interfered with the determination of the sulfanilamide. Such concentrations of thiocyanate occurred only after repeated injections in the dehydrated animal. Hence, in order to verify the data on total body water, duplicate experiments were done on several animals, one time using NaSCN but no sulfanilamide and the next time, sulfanilamide but no thiocyanate (see table 1). In the second experiment the period of dehydration was terminated when the weight loss was equivalent to that recorded in the first experiment. That comparable degrees of dehydration were reached is substantiated by the calculations of total body water and extracellular fluid derived from the electrolyte data. For these reasons it seemed justifiable to combine the data from two such experiments.

Dehydration by diuresis. The animals were weighed, placed on an animal board and catheterized to facilitate continuous collection of urine. Blood samples were taken for the determination of plasma volume, protein concentration, hematocrit value, available fluid (SCN), total water and electrolytes. After the control observations were made, a 50-per cent solution of sucrose was injected intravenously. A total amount equivalent to 20 cc/kgm. was allowed to run from a burette at the approximate rate of 2 to 3 cc. per minute through a hypodermic needle inserted in one of the small leg veins. The animals were then placed in metabolism cages to collect the urine and feces. Fifteen hours later the observations were repeated.

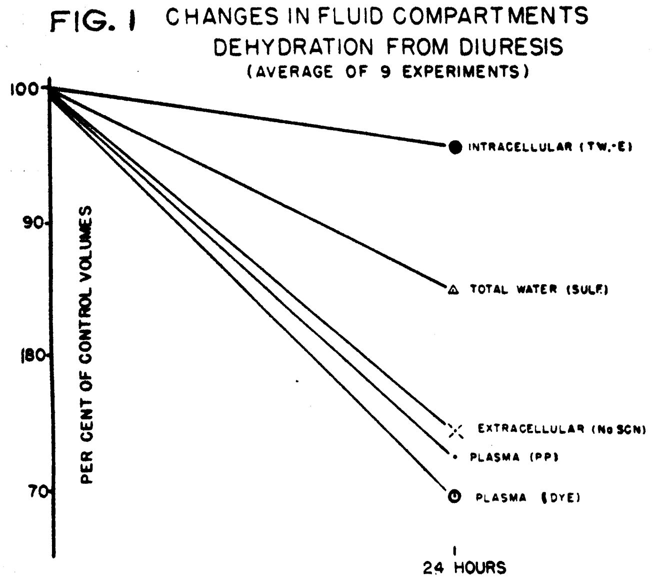
Dehydration by food and water deprivation. Control measurements were made of weight, plasma volume, available fluid (SCN), total water and electrolytes. The dogs were then placed in metabolism cages, the above measurements being repeated on the 5th and 11th days of the dehydration period. In four experiments complete electrolyte balance studies were made.

RESULTS

Figure 1 shows diagrammatically the average per cent reduction in plasma volume (T-1824), extracellular fluid (NaSCN) and total body water (sulfanilamide) observed after 24 hours in 9 experiments on dogs dehydrated by sucrose diuresis. Figure 2 illustrates the average changes on the 5th and 11th days in 12 experiments on dehydration produced by food and water deprivation. The changes in the plasma volume as calculated from the alteration in serum protein concentration are included as well as those based on the dye determinations. Table 1 shows a typical sample of the data from which figures 1 and 2 were constructed. The average volume changes in the fluid compartments are shown in table 3.

In the dehydration produced by the intravenous injection of sucrose, the weight loss ranged from 8 to 12 per cent. In the experiments on food and water deprivation the animals had lost 9 to 12 per cent of their body weight on the 5th day and 15 to 20 per cent on the 11th day. It should be noted that the weight loss after the diuresis is comparable to that observed after 5 days of water deprivation. For a detailed comparison of the water and electrolyte changes resulting from the two types of dehydration see below.

Total water loss. The weight loss in these experiments is a result not only of water loss but also of some solids. The sulfanilamide method enables one to



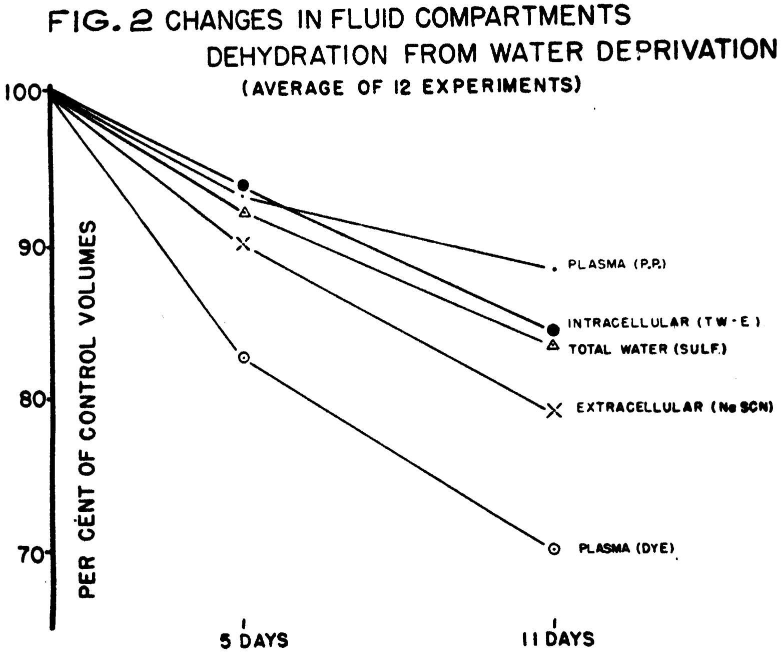


Fig. 1. Decrease in the size of the three fluid compartments in dehydration produced by diuresis (intravenous injection of 20 cc. per kgm. of 50 per cent sucrose solution).

Fig. 2. Decrease in the three fluid compartments after 5 and 11 days of food and water deprivation.

differentiate these two factors. In diuresis dehydration the water loss accounted for 90 per cent of the weight loss, whereas on the 11th day of water deprivation the total water loss was only 60 per cent of the decrease in weight (table 4a). These findings, estimated from the sulfanilamide method, were in agreement with the water loss calculated from the changes in the electrolyte balance according to the formula of Lavietes *et al.* (16). In the experiments on diuresis dehydration the largest difference in the results obtained by these two procedures was less than 10 per cent, the average difference only 2 per cent (table 3). The same close agreement of these two methods for calculating total water loss was observed in three of the four water-deprivation experiments in which complete electrolyte data were obtained.

TABLE 2. ELECTROLYTE CHANGES IN THE TWO TYPES OF DEHYDRATION FOR DOG J (SEE TABLE 1)

The intracellular water loss calculated from these data is compared with the intracellular loss obtained from the difference between total water loss (sulfa) and extracellular loss determined either by the thiocyanate method or by the sodium balance.

DEHYDRATION	SERUM				URINE LOSS				INTRACELLULAR LOSS		
	Sodium, mEq/L. H_2O		Chloride, mEq/L. H_2O		Sodium, mEq.	Chloride, mEq.	Potassium, mEq.	Nitrogen, mEq.	Calculated from electrolyte bal.	T.W.-Ex (SCN)	T.W.-Ex. (Na)
	Control	Dehydrated	Control	Dehydrated							
Diuresis									*cc.*	*cc.*	*cc.*
7-11-39	160.4	178.0	106.4	126.4	32.7	35.1	4.4	—	231	—	300
7-25-39	160.6	178.5	114.3	122.6	36.9	39.7	4.8	—	251	60	270
Water deprivation (5 days)											
11- 4-41	158.2	159.8	112.6	—	33.9	—	25.0	13.04	240	—	170
12-16-41	147.8	148.4	107.0	110.0	29.7	21.6	27.4	13.22	233	60	60
(11 days)											
11-10-41	158.2	173.2	112.6	124.0	33.9	16.1	56.3	27.71	698	—	590
12-22-41	147.8	158.0	107.0	117.2	35.0	27.7	65.4	27.10	640	460	610

TABLE 3. COMPARISON OF THE AVERAGE VOLUME CHANGES IN THE THREE FLUID COMPARTMENTS IN THE TWO TYPES OF DEHYDRATION

	DIURESIS	WATER DEPRIVATION	
		5 days	11 days
Number of experiments	9	10	14
Weight loss-grams	1203	1424	2575
Total water loss (Sulfa)-cc.	1106[1]	645	1623
Extracellular loss (SCN)-cc.	1019	386	920
Intracellular loss (Sulfa)-cc.	87	259	703
Plasma loss (T-1824)-cc.	194	97	193

[1] The corresponding figure for total water loss calculated from the electrolyte balance was 1089 cc.

Extracellular water loss. The extracellular fluid determined by the thiocyanate method was 25 per cent below the control in the diuresis experiments and was reduced 21 per cent on the 11th day of water deprivation (figs. 1 and 2). These reductions appear even more striking when compared with the total water loss. The average extracellular fluid loss was 85 per cent of the total water loss in the diuresis experiments, whereas in the water deprivation it was 67 and 57 per cent, respectively, for the 5th- and the 11th-day periods (see table 4b and *Discussion*).

It may be seen from table 4b that in the diuresis experiments the extracellular reduction, as calculated from the changes in sodium and chloride balance, gives a ratio of extracellular loss to total water loss of 71 per cent for sodium and 73 per cent for chloride. Calculations from the four water-deprivation experiments in which complete balance studies were done reveal that the ratio of extracellular loss to total water loss was on the average 81 and 82 per cent for sodium and chloride, respectively, on the 5th day, and 45 and 43 per cent, respectively, on the 11th day (table 4b). In these four experiments the corresponding values based on thiocyanate measurements were 90 and 58 per cent on the 5th and 11th days, respectively.

TABLE 4. INTERRELATIONSHIPS OF TOTAL WATER LOSS TO A) WEIGHT LOSS, B) EXTRACELLULAR LOSS AND C) INTRACELLULAR LOSS

	DIURESIS	WATER DEPRIVATION	
	per cent	*per cent*	*per cent*
(a) T.W. (Sulfa)/Wt.	90.4	44.5	60.1
(b) Extracell. (SCN)/T.W. (Sulfa)	84.8	67.0	56.7
(b) Extracell. (Na)/T.W. (Sulfa)	70.5	80.6[1]	44.8[1]
(b) Extracell. (Cl)/T.W. (Sulfa)	73.3	81.8[1]	43.1[1]
(c) Intracell. (SCN)/T.W. (Sulfa)	15.0	33.0	43.6
(c) Intracell. (Na)/T.W. (Sulfa)	29.5	19.4[1]	55.2[1]

Intracellular loss is expressed as the difference between total water loss (using sulfanilamide measurements) and extracellular loss (using thiocyanate measurements) or is calculated from sodium balance.

[1] Four experiments.

Plasma volume reduction. It can be seen (figs. 1 and 2) that the average plasma volume reduction determined by the dye method was 30 per cent after diuresis dehydration and 17 and 29 per cent, respectively, on the 5th and 11th day of water deprivation. Our results indicate that in both types of dehydration the plasma volume reduction is roughly proportional to the amount of fluid lost from the extracellular compartment (fig. 3). There is, however, no indication that the plasma volume is being maintained at the expense of the interstitial compartment. On the contrary the per cent reduction in plasma volume is in many instances considerably greater than the decrease in the extracellular fluid volume (SCN) (fig. 3).

Had we estimated the plasma volume reduction merely from the change in concentration of the serum proteins or from the change in the hematocrit value, different conclusions would have been drawn. In five days of water deprivation, the average plasma volume reduction calculated from the plasma protein change was 7 per cent and from the dye measurements, 17 per cent. In 11 days the average reduction calculated from the protein changes was 12 per cent and from the dye, 29 per cent (fig. 2). Indeed, in the last three experiments shown in table 1, the serum protein concentration actually went down, implying an increase in plasma volume!

However, in diuresis dehydration there was a fair agreement between these two methods of determining the reduction in plasma volume (fig. 1). For example, the reduction determined by the dye method was 30 per cent, whereas that estimated from the change in serum protein concentration was 27 per cent.

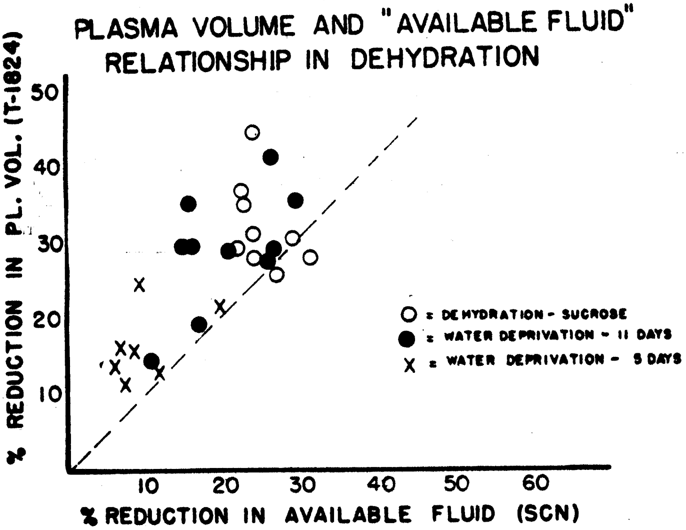


FIG. 3. GRAPH SHOWING THE RELATIONSHIP between the percentage reduction in plasma volume and available fluid (thiocyanate measurements) in dehydration.

Calculations of the decrease in plasma volume were also estimated from the change in hematocrit values. In diuresis dehydration, where the average reduction determined by the dye method was about 30 per cent, the reduction calculated from the hematocrit value was only 16 per cent, ranging from an apparent increase of 4 per cent to a decrease of 34 per cent. After water deprivation the average calculated reduction according to the hematocrit values was 7.6 per cent (5-day period) and 15.6 per cent (11-day period), as compared with values of 17.4 and 29 per cent obtained by the dye method. The range of values was as great as in the experiments on diuresis. The discrepancies cannot be attributed to the spleen for they were equally pronounced in experiments on five splenectomized dogs.

Intracellular water loss. Intracellular water has been calculated as the difference between the volumes of distribution of sulfanilamide and NaSCN. In the water-deprivation experiments the reduction of intracellular water was 6.5 and 16 per cent on the 5th and 11th days, respectively. Of the total water lost, the intracellular compartment contributed on the average 33 per cent by the 5th day and 44 per cent by the 11th day (see table 4c). Thus, as the degree of dehydration increases, the intracellular compartment gives up relatively more of its water. In the diuresis dehydration, however, the reduction in intracellular fluid volume was only 4.5 per cent, which constitutes 15 per cent of the total water lost. These findings are consistent with the electrolyte changes. The urinary loss of potassium in the diuresis dehydration was only 4 to 5 meq., while in 11 days of food and water deprivation the loss was 55 to 65 meq. (table 2). It should be noted that had we used the extracellular volumes calculated from either the sodium or chloride balance, instead of the thiocyanate values, the estimated intracellular loss would have been larger (see table 4c).

In four experiments on water deprivation the intracellular loss was calculated from the analyses of potassium and nitrogen in urine and from the changes in serum electrolyte concentration according to the formulae of Peters and Van Slyke (17) and Elkinton and Taffel (4).[3] The total intracellular loss estimated in this manner was larger than the value obtained by the dilution methods (Sulfa-SCN) but in closer agreement with that obtained by subtracting the extracellular loss calculated from the sodium balance from the total water loss determined either with the sulfanilamide method or from the total base exchange. This is illustrated in table 2, which shows the intracellular loss calculated by these three methods for *dog J*.

DISCUSSION

Waterhouse and Shannon (24), on the basis of tissue analyses, have questioned the use of sulfanilamide as a measure of total body water. However, in the unanesthetized dog this method was shown to give values in close agreement with those obtained from the volume of distribution of urea or from desiccation (9). Our findings in the dehydrated animal further confirm the usefulness of this method, since the changes in total body water determined by sulfanilamide were in close agreement with those calculated from the electrolyte balance (table 3).

Our results support Gamble's (1, 19) concept of the relation between the nature and amount of base excreted and the change in size of the intracellular and extracellular compartments. For example, when the sodium excretion is large in comparison to that of potassium, as in diuresis dehydration, the extracellular compartment suffers a greater loss than the intracellular, whereas when potassium excretion increases in the late stages of water deprivation, there is a corresponding decrease in the size of the intracellular compartment. It should be noted that the electrolyte changes we have observed after 5 days of water deprivation are in agreement with those reported by Winkler *et al.* (30) and by Black *et al.* on man (21).

On the whole the volume changes determined by the dilution methods (sulfanilamide and thiocyanate) correspond fairly well with the total water loss as well as with the reduction in intra- and extracellular water as calculated from the electrolyte balance. It will be noted that the fluid loss calculated from thiocyanate was somewhat greater than that calculated from either the sodium or chloride balance. This finding suggests that thiocyanate may be distributed in a larger volume of fluid than either Na or Cl. Winkler *et al.* (20) have demonstrated in the dog that thiocyanate, radioactive sodium and radioactive chloride are distributed through volumes equal to 36, 28 and 25 per cent of the body weight, respectively. Their results, however, are not confirmed by Kaltreider and his associates (22) on man, nor by Brues *et al.* (23) on the dog. In spite of these discrepancies in the absolute values obtained, the thiocyanate method is nevertheless useful in estimating the direction and order of magnitude of the changes in extracellular fluid volume.

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[3] The values for muscle potassium and nitrogen were obtained from Muntwyler's series of 24 normal dogs (18).

The observations reported by Darrow (27, 28) indicate that another factor should be considered in calculating fluid changes from sodium and potassium data; namely, that sodium may shift intracellularly. In reviewing our data with this point in mind, we found no indication that such a shift occurred in either type of dehydration. However, during recovery from water deprivation the drop in serum sodium concentration was greater than could be accounted for by the increase in extracellular volume (26). This change was of the order of magnitude represented by the amount of sodium in 100 to 400 cc. of extracellular fluid and a shift of sodium intracellularly as suggested by Darrow (27) would explain the apparent loss of sodium ion.

Evidence has been presented to show that in both types of dehydration the per cent reduction in plasma volume calculated from the dye method is usually greater than the per cent reduction in extracellular fluid (fig. 3). This is in agreement with the findings of Mellors *et al.* (6).

It should be emphasized that in water deprivation (see fig. 2) the reduction in plasma volume determined by the dye method is much greater than that calculated from the protein changes. In this situation the change in protein concentration is not a dependable measure of the change in plasma volume. Similar observations have been made by Glickman and co-workers (25) and Mellors *et al.* (6). In diuresis dehydration, however, where there is little opportunity for development of a negative nitrogen balance, the protein change gives a truer picture of the extent of reduction in plasma volume (fig. 1). These findings suggest that protein changes are a valid index of plasma volume change only when the experiment is of short duration and is uncomplicated by malnutrition.

In both types of dehydration the hematocrit changes were totally unreliable and often entirely misleading as evidence of reduction in plasma volume. However, the spleen cannot account for these discrepancies, since the changes in hematocrit value were no more consistent in the splenectomized animals than in the series of normal animals.

CONCLUSIONS

Data are presented from 9 experiments on dogs in which rapid dehydration was produced by sucrose diuresis and from 12 experiments in which gradual dehydration was produced by food and water deprivation. In the former the weight loss ranged from 8 to 12 per cent of the body weight. In the latter the weight loss at 5 days was 9 to 12 per cent and at 11 days, 15 to 20 per cent of the body weight.

In dehydration from diuresis the water loss constitutes 90 per cent of the weight loss, whereas in water deprivation it constitutes 45 per cent of the weight loss after 5 days and 60 per cent after 11 days (table 4a). In the diuresis dehydration the extracellular fluid loss (NaSCN) was equivalent to 84 per cent of the total water loss, whereas in water deprivation it was 67 per cent at 5 days and 57 per cent at 11 days (table 4b). The intracellular water loss was 15 per cent of the total in diuresis dehydration and 33 and 43 per cent, respectively, on the 5th and 11th days of water deprivation (table 4c). These differences in intracellular and extracellular water loss are reflected in the urinary excretion of potassium and sodium (tables 1 and 2).

The sulfanilamide method gave values for total water loss similar to those calculated from the electrolyte balance (see table 3). The extracellular loss determined by the thiocyanate method gave somewhat higher values than those calculated from the sodium and chloride balance (table 4b).

The plasma volume (T-1824) was reduced 30 per cent (average) in the dehydration with diuresis. In water deprivation the average reduction was 17 per cent on the 5th day and 29 per cent on the 11th day. There was no indication that in dehydration the plasma volume is maintained at the expense of the interstitial fluid volume. On the contrary the percentage reduction in plasma volume is generally greater than the percentage reduction in extracellular fluid volume (fig. 3).

The change in plasma protein concentration is a fair index of reduction in plasma volume after diuresis dehydration but not during water deprivation (fig. 1 and 2). The hematocrit changes were found to be utterly misleading as an index of volume reduction.

REFERENCES

1. Gamble, J. L., G. S. Ross and F. F. Tisdall. J. Biol. Chem. **57:** 633, 1923.
2. Kerpel-Fronius, E. Zeitschr. F. Kinderh. **57:** 489, 1935.
3. Nadal, J. W., S. Pedersen and W. G. Maddock. J. Clin. Invest. **20:** 691, 1941.
4. Elkinton, J. R. and M. Taffel. J. Clin. Invest. **21:** 787, 1942.
5. Gregersen, M. I. and E. E. Painter. This Journal **126:** 511, 1939.
6. Mellors, R. C., E. Muntwyler, F. R. Mautz and W. E. Abbott. J. Biol. Chem. **144:** 785, 1942.
7. Hopper, Jr., J., J. R. Elkinton and A. W. Winkler. J. Clin. Invest. **23:** 111, 1944.
8. Gregersen, M. I. and J. D. Stewart. This Journal **125:** 142, 1939.
9. Painter, E. E. This Journal **129:** 744, 1940.
10. Neuhausen, B. S. and D. M. Rioch. J. Biol. Chem. **55:** 353, 1923.
11. Barbour, H. G. and W. F. Hamilton. J. Biol. Chem. **69:** 625, 1926.
12. Butler, A. M. and E. Tuthill. J. Biol. Chem. **93:** 171, 1931.
13. Shohl, A. T. and H. B. Bennett. J. Biol. Chem. **78:** 643, 1928.
14. Saifer, A. and M. Kornblum. J. Biol. Chem. **112:** 117, 1935.
15. Van Slyke, D. D. J. Biol. Chem. **58:** 523, 1923.
16. Lavietes, P. H., L. M. D'Esopo and H. E. Harrison. J. Clin. Invest. **14:** 251, 1935.
17. Peters, J. P. and D. D. Van Slyke. Quantitative Clinical Chemistry. Interpretations. Baltimore: Williams & Wilkins, 1932. Vol. I.
18. Muntwyler, E., R. C. Mellors and F. R. Mautz. J. Biol. Chem. **134:** 345, 1940.

19. Gamble, J. L. Chemical Anatomy, Physiology and Pathology of Extracellular Fluid. A lecture syllabus. 1942.
20. Winkler, A. W., J. R. Elkinton and A. J. Eisenman. This Journal **139:** 239, 1943.
21. Black, D. A. K., R. A. McCance and W. F. Young. J. Physiol. **102:** 406, 1944.
22. Kaltreider, N. L., G. R. Meneely, J. R. Allen and W. F. Bale. J. Exp. Med. **74:** 569, 1941.
23. Brues, A. M., W. E. Cohn, S. S. Kety, I. T. Nathanson, A. L. Nutt, D. M. Tibbetts, P. C. Zamecnik and J. C. Aub. J. Clin. Invest. **24:** 835, 1945.
24. Waterhouse, A. and J. A. Shannon. Proc. Soc. Exp. Biol. and Med. **50:** 189, 1942.
25. Glickman, N., F. K. Hick, R. W. Keeton and M. M. Montgomery. This Journal **134:** 165, 1941.
26. Holmes, J. H. and E. E. Painter. To be published.
27. Darrow, D. C. New Eng. J. Med. **233:** 91, 1945.
28. Darrow, D. C. J. Pediat. **28:** 515, 1946.
29. Peters, J. P. and D. D. Van Slyke. Quantitative Clinical Chemistry. Methods. Baltimore: Williams & Wilkins, 1932. Vol. II.
30. Winkler, A. W., T. S. Danowski, J. R. Elkinton and J. P. Peters. J. Clin. Invest. **23:** 807, 1944.

EFFECTS OF ISOTONIC SOLUTIONS AND OF SULFATES AND PHOSPHATES ON THE DISTRIBUTION OF WATER AND ELECTROLYTES IN HUMAN BLOOD[1]

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In previous articles (1, 2) evidence was presented that the addition to human blood of an isotonic solution of sucrose, to which the blood cells are impervious, caused no demonstrable transfer of electrolytes between cells and serum, although concentration gradients of Na and K and distribution coefficients of H^+, HCO_3^- and Cl^- are profoundly altered by this procedure. An investigation of the effects of other isotonic solutions on the distribution of electrolytes has, therefore, been carried out. At the same time the effects of adding sodium and potassium salts with anions that do not cross the membranes of the resting cells have been studied. For this purpose phosphates and sulfates were selected.

EXPERIMENTAL METHODS

Defibrinated blood, with and without additions of solutions or salts, was analyzed after equilibration at 37°C. with an atmosphere of 40 mm. of CO_2 in air. The technical procedures have been described in previous articles (1, 2). The materials added were: *a*) isotonic solutions of sucrose, sodium chloride, potassium chloride, sodium sulfate, potassium sulfate, sodium phosphate and potassium phosphate; *b*) solid sodium sulfate and potassium sulfate; and *c*) hypertonic solutions of sodium phosphate and potassium phosphate. The phosphate solutions were prepared in such a manner that the ratio, dibasic salt: monobasic salt, equalled 4:1. The amount of solution added was, in every instance, equal to one half the volume of blood to which it was added. In the experiments with solid sulfate enough salt was added to yield a concentration in the whole blood of 93.3 millimols, or 140 milliosmols.

Since the blood was defibrinated in open vessels and was equilibrated only once in order to minimize hemolysis, the tension of CO_2 finally attained was not precisely 40 mm. It differed from 40 mm. by the quantity of CO_2 absorbed or given off by the blood in the tonometers during the process of equilibration. Moreover, since treated and untreated samples did not, in the process of equilibration, take up or give off the same quantities of CO_2, the tensions of CO_2 in these solutions were not quite identical. In order to determine the magnitude of the error introduced by these factors in four of the experiments a third sample of blood was placed in a sampling bulb over mercury just before equilibration. From the difference between the CO_2 in this sample and that in the equilibrated samples the change of CO_2 tension during equilibration can be calculated. The results are presented in table 1. Since the volumes of the tonometers were large in proportion to the volumes of blood used, the errors from this source were, in most instances, negligibly small. There is some doubt about the preparation of the gas mixtures in *experiment 3*, which was chronologically the first of the series.

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[1] Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

Two experiments were planned to ascertain whether or not the process of equilibration per se altered the nature and distribution of phosphorus compounds. For this purpose one sample of defibrinated blood was preserved anaerobically while another was equilibrated in the usual manner. The results are presented in table 2. In neither experiment did the phosphates change appreciably.

TABLE 1. DEVIATIONS OF CO_2 TENSION ARISING FROM INCOMPLETE EQUILIBRATION
CO_2 tension of untreated and treated blood should equal 40 mm. Hg

	EXPERIMENT NUMBER			
	1	3	4	5
CO_2 in original blood, vol. %	43.5	52.4	50.9	46.0
CO_2 of untreated blood, vol. %	49.4	37.1	43.9	43.8
CO_2 of treated blood, vol. %	32.8	37.2	35.1	33.7
CO_2 tension of untreated blood, mm. Hg	37.3	47.2	43.3	41.1
CO_2 tension of treated blood, mm. Hg	38.3	39.0	39.5	38.1

TABLE 2. EFFECT OF EQUILIBRATION ON THE PHOSPHATES OF CELLS AND SERUM

EXPERIMENT	SERUM		PHOSPHORUS, MILLIMOLS PER LITER					
	CO_2 mEq. per liter	pH	Inorganic			Organic		
			Blood	Serum	Cells	Blood	Serum	Cells
1. Original blood	29.8	7.48	0.95	1.11	0.75	6.84	0.03	14.9
Equilibrated blood	25.2	7.40	0.99	1.12	0.84	6.80	0.07	14.8
2. Original blood	29.8	7.48	0.85	1.18	0.49	6.49	0.06	13.7
Equilibrated blood	27.2	7.44	0.93	1.25	0.58	6.41	0.07	13.4

It has already been demonstrated by Bourdillon (3) and others (4, 5) that the membranes of the blood cells are impervious to the sulfate ion. Nevertheless, because this has been disputed and because the concentrations of sulfate produced in these experiments are higher than those studied by most observers, this point was again examined. Cell volume and serum sulfate were measured before and after the addition of sodium sulfate and potassium sulfate in quantities as large as those used in the present experiments. For the measurement of sulfate a gravimetric benzidine sulfate method was derived from a combination of Power and Wakefield's (6) procedure and Hald's (7) gravimetric technique for the measurement of total base of serum. From table 3 it is apparent that no detectable amounts of sulfate enter the cells after addition to blood of large amounts of sodium or potassium sulfate.

CALCULATIONS

In all but 2 of the 12 experiments the proportions of water in cells and serum were measured by weighing blood and serum before and after drying to constant weight at 95°C. In the remaining two water was estimated from total nitrogen by the formula of Eisenman, Mackenzie and Peters (8). Cell volumes were measured by hematocrit. When solutions were added to blood it was possible to estimate dilution more precisely from the relative volumes of blood and solution than from measurements of cell volume and solids. Likewise, in estimating the effects of increments of salts, the amounts added, which could be measured with great precision, were more reliable than analytical figures. This subject has been discussed in a previous paper (2). The analytical data from the untreated whole blood were, therefore, accepted, but the concentrations in the treated blood were calculated from these. The actual analytical data from the treated whole blood served as a check against gross errors. The percentage differences between the calculated values and the actual measured values are shown in table 4. In addition to water various quantities of solids were added to the treated samples. Since the amount of water in these samples was estimated from the weight of solids before and after drying, these solid increments must theoretically be taken into account. The first two columns represent the differences between observed values for water in treated whole blood and values calculated with and without consideration of the solid increments. Only in the sucrose experiments in which the quantities of added solids were particularly large do the solids make any appreciable difference. In most cases the differences between calculated and measured values are well within the experimental error. It must be recognized that theoretically there is as great a probability of error in the analysis of the untreated as of the treated blood and that these errors may fall in opposite directions, thereby exaggerating the error in the increment. The measured water of blood and serum in the sucrose experiments is erroneously high because these samples charred when heated. These are the only experiments in which the calculated rather than the measured value was used for serum. The large amounts of added phosphate caused some difficulty in the

TABLE 3. EFFECT OF ADDING ISOTONIC SODIUM AND POTASSIUM SULFATE TO BLOOD

In each experiment an amount of isotonic sulfate was added which contained 100 mEq. of sulfate. The total amount in the serum is expressed in mEq. per liter of original whole blood. Therefore, if all the sulfate remains in the serum, the total in treated serum should equal 100 mEq. + the total in control serum.

EXPERIMENT	CONTROL SERUM			TREATED SERUM		
	Volume per cent	SO_4 mEq. per liter	SO_4 mEq. total	Volume per cent	SO_4 mEq. per liter	SO_4 mEq. total
1. Na_2SO_4	51.2	0.7	0.4	68.2	95.5	99.9
2. K_2SO_4	53.2	0.0	0.0	69.3	94.9	100.3
3. K_2SO_4	50.5	1.3	0.7	67.0	99.6	100.1

measurement of the sodium and potassium of whole blood in *experiments 7* and *8*. In the sodium phosphate experiments the values for sodium in the treated sera were evidently too large, since they gave negative values for cell sodium. They were, therefore, arbitrarily reduced by one or two millimols to make them agree more closely with calculated values. This correction, which is within the experimental error, yielded values for cell sodium slightly above zero. In the experiment with solid potassium sulfate, the measured sodium of the treated whole blood was 9.8 per cent above the calculated figure, which probably indicates that this sample became contaminated in some manner. In this instance also a correction of two millimols was applied in order to yield a positive instead of a negative value for cell sodium. The error in total phosphorus exceeded 3 per cent twice, once when sucrose made ashing difficult and once when there was a large amount of added phosphate. The inorganic phosphorus of serum was considered more reliable than the total phosphorus in the sucrose and the phosphate experiments: in the former because of the difficulty of ashing serum containing large amounts of sucrose; in the latter because the proportion of organic phosphate was negligible.

TABLE 4. DIFFERENCE BETWEEN CALCULATED AND MEASURED VALUES FOR VARIOUS COMPONENTS OF TREATED BLOOD

All differences are expressed as measured−calculated values in per cent of the total quantity of a component in whole blood.

EXPERIMENT	WATER		Cl	TOTAL P	Na	K	VOLUME OF SERUM
	Corrected for added solids	Not corrected for added solids					
1. Isotonic sucrose	+1.9[1]	−1.4	−0.4	+1.7	0	−2.0	+1.2
2. Isotonic sucrose	+1.5[1]	−2.0	−0.6	+3.8	+0.6	+0.7	+0.3
3. Isotonic NaCl	−0.5	−0.8	0[2]	−2.1	−0.1[2]	+2.7	+0.9
4. Isotonic KCl	−0.8	−1.2	+0.3	−0.7	+3.6	+1.8	−0.5
5. Isotonic Na_2SO_4	+0.3	−0.1	+1.1	+1.7	−1.4	−1.9	+1.1
6. Isotonic K_2SO_4	[3]	−0.6	−0.7	+1.2	+3.3	+1.6	+3.6
7. Isotonic Na-phosphate	0.0	−0.5	−0.6	+3.5	−5.1	+5.9	−0.3
8. Isotonic K-phosphate	0.0	−0.6	−0.2	−1.3	+4.5	−3.5	+0.7
9. Hypertonic Na-phosphate	+0.3	−0.6	−1.3	−1.2	−1.4	−2.6	[5]
10. Hypertonic K-phosphate	−0.5	−1.4	−1.5	+0.3	−1.2	−1.1	[5]
11. Solid Na_2SO_4	0.0	−0.8	−0.2	−0.5	0	−0.6	[5]
12. Solid K_2SO_4	[3]	+0.6	−0.4	+2.4	(+9.8)[4]	+0.8	[5]

[1] Charring occurred in drying these specimens.

[2] A value of 79.6 instead of 74.6 was used for sodium chloride added in this case.

[3] The solids were not considered in these experiments because the water was estimated from the total N of blood.

[4] This sample must have been contaminated.

[5] Measured and not calculated values had to be used because there was a shift of water in these experiments from cells to serum.

Obviously analytical values for CO_2 were used throughout. It is assumed in the calculation of bicarbonate and pH that the addition of solutions and salts did not alter the solubility of CO_2 or the value of pH.

By careful analysis of the data it will be found that none of the errors mentioned affect the validity of these experiments nor the conclusions derived from them.

TABLE 5. THE EFFECTS OF ADDING ISOTONIC SOLUTIONS, HYPERTONIC PHOSPHATE AND SOLID SULFATE TO BLOOD

Original data

EXPERIMENT	TREATMENT OF BLOOD	CELL VOLUME	WATER		CO_2		Cl		Na		K		PHOSPHORUS			
													Inorganic		Organic	
			Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum
		%	%	%	vol. %	vol. %	mM./l.	mM./l.	mM./l.	mM./l.	mM./l.	mM./l.	mM./l.	mM./l.	mM./l.	mM./l.
1	Untreated blood	37.3	85.8	94.1	49.4	55.1	80.8	98.2	86.4	134.5	44.1	4.0	0.96	1.06	7.60	0.06
	Isotonic sucrose	24.9	87.5	92.7	32.8	33.1	53.8	55.0	57.6	73.7	29.4	2.4	0.62	0.60	5.09	[1]
2	Untreated blood	54.1	80.8	92.1	49.4	63.5	72.8	98.2	74.4	142.2	61.8	5.8	0.90	1.49	10.61	0.05
	Isotonic sucrose	36.1	84.2	91.5	32.5	31.5	48.5	48.8	49.6	66.6	41.2	3.1	0.60	0.59	7.08	[1]
3	Untreated blood	51.3	81.9	92.1	37.1	47.1	70.4	100.8	79.0	139.0	55.0	4.8	0.54	0.87	7.82	0.05
	Isotonic NaCl	34.2	87.6	96.3	37.2	41.7	100.0	120.4	105.7	147.0	36.7	2.6	0.54	0.56	5.03	0.0
4	Untreated blood	45.2	82.4	93.5	43.9	53.9	82.0	108.8	84.0	149.3	49.0	4.5	0.90	1.07	6.60	0.0
	Isotonic KCl	30.1	87.9	95.2	35.1	40.0	104.7	124.2	56.0	79.6	82.7	74.6	0.67	0.68	4.33	0.04
5	Untreated blood	50.6	81.2	92.3	43.8	53.9	81.0	107.8	77.1	145.0	53.5	4.4	0.66	0.87	7.90	0.08
	Isotonic Na_2SO_4	33.7	87.0	96.1	33.7	37.6	54.0	63.8	118.1	172.1	35.7	2.5	0.37	0.42	5.34	0.05
6	Untreated blood	44.0	84.3	93.0	44.5	53.5	81.0	99.6	91.5	132.5	47.6	5.1	1.07	1.32	8.21	0.12
	Isotonic K_2SO_4	29.3	89.6	95.7	32.8	35.3	54.0	61.0	61.0	75.1	98.4	95.1	0.48	0.56	5.70	0.06
7	Untreated blood	46.6	82.05	92.3	45.6	57.3	76.6	99.4	71.6	133.3	50.5	4.1	0.82	0.93	8.18	0.04
	Isotonic Na-phosphate	31.1	87.6	95.1	35.8	38.1	51.1	51.8	107.7	156[3]	33.7	2.3	35.3	45.8	4.9	0[2]
8	Untreated blood	42.4	83.8	92.5	45.2	53.9	86.6	107.6	83.5	141.5	45.7	5.0	0.77	1.02	6.51	0.06
	Isotonic K-phosphate	28.3	88.6	95.4	35.0	37.3	57.7	63.6	55.7	77.6	94.8	89.0	35.8	45.0	4.7	0[2]
9	Untreated blood	46.6	81.8	92.3	45.6	55.5	81.4	102.6	78.4	141.4	51.5	3.6	0.93	1.04	7.01	0.07
	Hypertonic Na-phosphate	26.4	87.1	95.6	33.9	36.6	54.3	60.2	151.9	206[3]	34.3	2.5	57.8	73.4	7.7	0[2]
10	Untreated blood	47.1	82.7	92.7	50.9	65.0	79.2	96.3	82.5	145.5	49.7	4.0	0.96	1.22	7.70	0.08
	Hypertonic K-phosphate	27.5	87.6	95.8	38.4	41.0	52.8	53.6	55.0	73.3	129.4	134.2	54.7	70.6	4.6	0[2]
11	Untreated blood	45.5	82.7	91.8	48.1	58.4	81.8	104.4	82.0	148.3	50.0	4.1	0.92	1.05	8.08	0.08
	Solid Na_2SO_4	34.7	82.1	93.2	48.4	52.5	81.8	100.8	175.3	261.6	50.0	5.0	0.62	0.72	8.38	0.04
12	Untreated blood	47.6	83.1	93.5	49.7	59.9	85.0	108.4	77.5	142.5	48.3	4.7	0.81	0.90	7.03	0.04
	Solid K_2SO_4	36.6	83.1	94.4	50.8	56.8	85.0	102.6	77.5[3]	122.2[3]	141.6	152.7	0.60	0.67	7.24	0.05

[1] Sucrose interfered with ashing.

[2] Organic acid soluble phosphate of serum too low to read in presence of such large amounts of inorganic phosphate.

[3] Sodium values corrected for reasons mentioned in text.

In experiments in which isotonic solutions were added to blood without exception the volumes of the blood cells remained unchanged. As far as water was concerned the effect of adding these solutions was only to dilute the serum. To the additions of solid sulfate and hypertonic phosphate solutions the cells behaved as if the added salts were completely dissociated into osmotically active ions and as if the cellular membranes permitted the unobstructed passage of water under the influence of osmotic pressure.

In only one experiment, *no. 6*, in which isotonic K_2SO_4 was added, was there a significant transfer of sodium or potassium. In this experiment sodium appeared to leave the cells. This might be attributed to some unrecognized technical error were it not that in *experiment 12*, in which solid K_2SO_4 was added, there is a similar transfer of sodium that is just within the limit of error of the method. Since potassium, not sodium, was added in these two experiments, the maximum error should not fall on sodium. In the comparable experiments, 5 and 11, with additions of sodium sulfate, there are no significant exchanges of either sodium or

TABLE 6. VALUES DERIVED FROM DATA IN TABLE 5

EXPERIMENT	DISTRIBUTION RATIOS							TOTAL AMOUNT IN A LITER OF BLOOD, OF							
	pH	HCO_3	Cl	P	Na + K	$\frac{D\ HCO_3}{D\ Cl}$		HCO_3		Cl	Na	K	Phosphorus		
													Inorganic		Organic
								serum	cells	cells	cells	cells	serum	cells	cells
								mEq.	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mM.*	*mM.*	*mM.*
1. Untreated blood	7.39	1.08	1.46	1.04	1.10	0.74		14.7	6.2	19.2	2.0	41.6	0.67	0.29	7.56
Isotonic sucrose	7.15	0.81	0.85	0.73	2.05	0.95		15.3	4.9	18.8	3.4	41.4	0.68	0.24	7.64
							change	+0.6	−1.3	−0.4	+1.4	−0.2	+0.01	−0.05	+0.08
2. Untreated blood	7.45	1.33	1.48	2.79	1.10	0.90		12.4	8.5	27.7	9.1	59.1	0.68	0.22	10.60
Isotonic sucrose	7.13	0.71	0.79	0.74	2.36	0.90		12.4	7.7	26.0	10.6	58.8	0.57	0.33	10.62
							change	0	−0.8	−1.7	+1.5	−0.3	−0.11	+0.11	+0.02
3. Untreated blood	7.32	1.37	1.90	3.13	1.11	0.72		9.6	5.8	21.3	11.3	52.7	0.43	0.11	7.80
Isotonic NaCl	7.26	1.08	1.46	0.84	1.17	0.74		17.2	6.1	31.2	13.5	52.4	0.56	0.25	7.55
							change	+7.6	+0.3	+9.9	+2.2	−0.3	+0.13	+0.14	−0.25
4. Untreated blood	7.38	1.28	1.62	1.15	0.95	0.79		12.5	6.0	22.4	2.2	46.5	0.59	0.31	6.60
Isotonic KCl	7.24	1.30	1.56	0.76	0.89	0.83		17.4	4.3	26.8	0.5	45.8	0.71	0.30	6.46
							change	+4.9	−1.7	+4.4	−1.7	−0.7	+0.12	−0.01	−0.14
5. Untreated blood	7.38	1.22	1.50	1.45	0.99	0.81		11.3	7.2	27.7	5.5	51.3	0.43	0.23	7.86
Isotonic Na_2SO_4	7.21	1.05	1.32	1.19	0.90	0.80		15.5	5.4	17.6	6.0	51.0	0.42	0.13	7.96
							change	+4.2	−1.8	−10.1	+0.5	−0.3	−0.01	−0.10	+0.10
6. Untreated blood	7.38	1.31	1.37	1.35	1.30	0.96		12.7	6.0	25.2	17.3	44.7	0.73	0.34	8.14
Isotonic K_2SO_4	7.18	1.08	1.29	1.51	1.00	0.84		15.5	4.7	16.3	11.9	46.8	0.59	0.13	8.49
							change	+2.8	−1.3	−8.9	−5.4	+2.1	−0.14	−0.21	+0.35
7. Untreated blood	7.41	1.40	1.50	1.03	1.00	0.93		13.0	6.2	23.5	0.4	48.3	0.50	0.32	8.16
Isotonic Na-phosphate	7.22	0.92	0.78	2.85	0.88	1.18		16.3	6.0	23.0	0.3	48.1	−3.9	5.6	7.3
							change	+3.3	−0.2	−0.5	−0.1	−0.2	−4.4	+5.3	−0.9
8. Untreated blood	7.38	1.29	1.44	1.77	0.93	0.90		13.2	5.9	24.6	2.0	42.8	0.58	0.19	6.48
Isotonic K-phosphate	7.21	0.96	1.11	2.70	0.88	0.87		16.6	5.1	18.2	0.1	46.5	−5.2	5.3	7.1
							change	+3.4	−0.8	−6.4	−1.9	+3.7	−5.8	+5.1	+0.6
9. Untreated blood	7.39	1.24	1.36	0.97	1.03	0.91		12.6	6.7	26.6	2.9	49.6	0.55	0.38	6.97
Hypertonic Na-phosphate	7.20	0.92	1.06	3.38	0.90	0.87		16.7	4.3	14.9	0.4	48.7	−9.3	5.7	11.6
							change	+4.1	−2.4	−11.7	−2.5	−0.9	−9.9	+5.3	+4.6
10. Untreated blood	7.47	1.46	1.23	1.39	0.98	1.19		14.7	6.9	28.3	5.5	47.6	0.64	0.32	7.66
Hypertonic K-phosphate	7.25	0.90	0.73	3.88	0.86	1.23		18.6	5.4	20.9	2.8	48.2	−3.5	5.2	6.9
							change	+3.9	−1.5	−7.4	−2.7	+0.6	−4.1	+4.9	−0.8
11. Untreated blood	7.42	1.30	1.49	1.07	0.90	0.87		13.6	6.8	24.9	1.2	47.8	0.57	0.35	8.03
Solid Na_2SO_4	7.37	0.84	1.44	1.08	0.84	0.58		14.5	6.0	16.0	4.5	46.7	0.47	0.15	8.36
							change	+0.9	−0.8	−8.9	+3.3	−1.1	−0.1	−0.2	+0.3
12. Untreated blood	7.43	1.21	1.40	0.96	0.90	0.86		13.4	7.7	28.2	2.8	45.8	0.47	0.34	7.01
Solid K_2SO_4	7.40	0.94	1.27	0.92	0.66	0.74		15.3	6.3	20.0	0	44.8	0.42	0.18	7.21
							change	+1.9	−1.4	−8.2	−2.8	−1.0	−0.05	−0.16	+0.20

potassium. With this exception the effects of potassium and sodium salts are indistinguishable. No sodium nor potassium enters the cells with the phosphate.

In all experiments with the exception of the first two, in which sucrose was added, the quantity of bicarbonate in the serum rises. Only a small part of this increment is derived from the cells, the bicarbonate of which changes only slightly. The source of the increment in the serum is the combination of CO_2 with base liberated from other anions. In the last two experiments in which no water was added and in which the changes of pH were minimal, the changes of bicarbonate in cells and serum were reciprocal and almost equal.

The sucrose experiments confirm the previous observations (1, 2). In spite of the fact that isotonic sucrose solution reduces the pH and the concentrations of all electrolytes in the serum, profoundly altering all concentration gradients and distribution coefficients, the composition of the contents of the cells is quite unaffected. There may have been a minimal loss of bicarbonate from the cells.

In the chloride experiments, 3 and 4, chloride enters the cells according to expectation, since the cell membranes are permeable to this ion. The bicarbonate of the cells, on the other hand, falls slightly in only one experiment, while serum bicarbonate increases in both.

The responses to isotonic sulfate differ radically from the responses to either sucrose or chloride. Large quantities of chloride escape from the cells together with small amounts of bicarbonate. In addition a small amount of extra serum bicarbonate is formed.

The reaction to isotonic phosphate is similar, but the changes of chloride and bicarbonate are smaller.

Hypertonic sulfate solutions caused large transfers of chloride from cells to serum but had little effect on bicarbonate. The reactions to hypertonic phosphate solutions had the same general character as those to isotonic solutions of the same salts, but the transfers of chlorides were larger.

Distribution coefficients of Cl and HCO_3 fell in every experiment as pH diminished. In most instances they fell proportionally. Usually, however, there was no consistency in these exceptions. For example, in *experiment 1* the distribution coefficient of Cl seems to fall more than that of HCO_3. In the companion sucrose *experiment 2* they retain their relations to one another with the greatest precision. The cause of the difference is the small transfer of HCO_3 in *experiment 1* and apparent variations of cell chloride that are within the experimental error. A similar discrepancy is seen in the two isotonic phosphate experiments, *7* and *8*. The only consistent departure from equality is in the two solid sulfate experiments, *11* and *12*, in both of which the distribution coefficient of bicarbonate falls far more than that of chloride. Changes of pH were minimal in these experiments.

DISCUSSION

The present series of experiments confirm the general impermeability of the membranes of the red blood cells to sodium and potassium. The possible exception of the potassium sulfate experiments deserves further inquiry. If sodium does emerge from the cells, as it seems to in response to the addition of potassium sulfate, its movement must be attributed to some peculiar influence of this salt upon the cells. The sodium moves against a large concentration gradient and, therefore, not by simple diffusion. The transfer cannot be attributed to a Gibbs-Donnan effect, because this should be equally evident in the sodium sulfate and in the phosphate experiments.

The impermeability of the blood cell membranes to sulfate has again been demonstrated. To the inorganic phosphate ion the impermeability appears to be less absolute. In earlier experiments by Halpern (9) inorganic phosphate seemed to be excluded from blood cells. In those experiments, however, the additions of phosphate were relatively small. Apparently when the concentration of phosphate in the serum is greatly elevated, a small amount traverses the membrane, without sodium or potassium. It will be shown (10) that this transfer is associated with a change in the metabolism of the cells and is not due merely to the high concentration gradient of phosphate.

The dilution of the serum with the isotonic solutions reduces the concentration of HCO_3 by about 50 per cent of its original value. Since the CO_2 tension (and consequently the concentration of H_2CO_3) remains unchanged, the pH falls. Base previously balanced by other buffer anions, therefore, is released to form bicarbonate. The cells contain far less 'combined CO_2', of which only a part is bicarbonate, and their contents are not diluted by the isotonic solutions as the serum is. Consequently they play a variable but minor part in the adjustment to the change of environment. When the cell volume was changed, as it was in the last two experiments in which salt without water was added and in which the changes of pH were minimal, the changes of bicarbonate in cells and serum were reciprocal and almost equal.

That isotonic sucrose should produce so little disturbance was at first unexpected; but, on consideration, should have been anticipated. Dilution of the serum at constant CO_2 lowers its pH greatly. If, however, there is no change of bicarbonate in the cells, the pH of their contents will remain constant. Since at constant CO_2 tension H^+ and bicarbonate are reciprocally related, dilution will change their concentrations and that of Cl^- in the serum proportionally. In the cells H^+, HCO_3^-, the inactive fraction of 'combined CO_2' and both inactive and active fractions of Cl^- will be unchanged. The distribution of coefficients of H^+, HCO_3^- and Cl^- will, therefore, be changed proportionally. Although all will fall, their relations to one another will remain the same.

In the chloride experiments a shift of Cl^-, to which the cell membrane is permeable, is to be expected.

Since phosphate is an excellent buffer, some increase in bicarbonate of serum is to be expected after its addition, which necessitates a transfer of chloride from cells to serum. Since sulfates are salts of a strong acid and both ions of these salts are excluded from the cells, it seemed possible that they would have an effect like that of sucrose. Instead they behave like phosphates. In response to the addition of isotonic sulfates, the bicarbonate of serum rises and chloride emerges from the cells. With a fall of pH no greater than that produced by sodium chloride and without the addition of buffer, enough base is apparently released from other buffers in the serum to balance not only the extra bicarbonate which is formed but also a large extra load of chloride derived from the cells. The total extra anion load in the serum in the two experiments was 14.3 and 11.8 milliequivalents. This is more, not less, than was transferred in the isotonic phosphate experiments. The increase of serum bicarbonate in this as in other experiments of the series must be chiefly a response to dilution. It is minimal after the addition of solid sulfate in which exchange of water as well as electrolytes involves only a redistribution of materials previously in the blood. The chloride shift is, however, of the same order of magnitude in both series of experiments.

The changes of pH, bicarbonate and chloride in the whole series are so small that the errors of distribution coefficients are considerable. In general they follow the principles previously described. The distribution coefficients of HCO_3 and Cl fall with the pH. The relation of pH to distribution coefficients in the two sucrose and four phosphate experiments are quite similar. The relation in the chloride and sulfate experiments is more irregular. Whether this deviation is significant or not, only further experiments can determine. In general, distribution coefficients of HCO_3 and Cl parallel one another. The only exception is the solid sulfate experiments to which attention was called above.

SUMMARY

Isotonic sucrose solutions and isotonic and hypertonic solutions of the sulfates and phosphates of sodium and potassium were added to human blood. No sodium, potassium nor sulfate crossed the cell membranes. Some phosphate entered the cells when phosphate was added to blood. Associated transfers of chloride and bicarbonate are discussed.

REFERENCES

1. Peters, J. P., M. Tulin, T. S. Danowski and P. M. Hald. This Journal **148:** 568, 1947.
2. Hald, P. M., M. Tulin, T. S. Danowski, P. H. Lavietes and J. P. Peters. This Journal **149:** 340, 1947.
3. Bourdillon, J. and P. H. Lavietes. J. Clin. Invest. **15:** 301, 1936.
4. Wiechmann, E. Arch. ges. Physiol. **189:** 109, 1921.
5. Woodhouse, D. L. and F. A. Peckworth. Biochem. J. **26:** 309, 1932.
6. Power, M. H. and E. G. Wakefield. J. Biol. Chem. **123:** 665, 1938.
7. Hald, P. M. J. Biol. Chem. **103:** 471, 1933.
8. Eisenman, A. J., L. B. Mackenzie and J. P. Peters. J. Biol. Chem. **116:** 33, 1936.
9. Halpern, L. J. Biol. Chem. **114:** 747, 1936.
10. Heinsen, A. J. This Journal **152:** 216, 1948.

EFFECT OF ACETYLCHOLINE, CAFFEINE AND ALKALOIDS ON ACTIVITY OF MUSCLE ADENOSINETRIPHOSPHATASE[1]

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It is a current belief that muscle contraction occurs through physical changes in the actomyosin system (1). The energy necessary to recharge the energy-poor contracted system to the energy-rich relaxed state is probably mainly supplied through the splitting of one phosphate group from adenosinetriphosphate (ATP) by the adenosinetriphosphatase activity of particles attached to myosin.

Acetylcholine in very low concentrations induces shortening of muscle. The experimental evidence for the action of acetylcholine on the isolated actomyosin system or its constituents is, however, restricted to the following: *a*) in the presence of acetylcholine, potassium may be released from actomyosin (2) and myosin (3); and *b*) acetylcholine does not induce shortening of isolated actomyosin fibers and does not modify the effect of ATP in inducing shortening of the actomyosin fibers (4).

An improvement in muscle function may be achieved not only by augmenting the contractile process in muscle but also be improvement in the recovery processes. In the following, evidence is presented that in the presence of acetylcholine the activity of muscle adenosinetriphosphatase is increased. The effects of other chemical agents known to modify the shortening of muscle (alkaloids, caffeine) on the activity of muscle adenosinetriphosphatase were also determined.

EXPERIMENTAL

Analytical procedures. Phosphorus was determined by the method of Youngburg and Youngburg (5). Nitrogen determinations were carried out by the procedure of Bock and Benedict (6).

Preparation of adenosinetriphosphate. The barium salt of ATP was prepared according to the method described by Needham (7) with minor modifications (8). The sodium salt was prepared from the barium compound by precipitation of the barium with the stoichiometric amount of sulfuric acid. The solution was treated with veronal buffer and the pH was adjusted to 8.5 with sodium hydroxide immediately before use, as determined by the glass electrode. The nitrogen to phosphorus and the 15-minute labile phosphorus to total phosphorus ratios for the preparations of ATP used in these experiments were as follows: N:P ratio was 5.04:3; ratio of labile to total P was 2.05:3.

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Preparation of adenosinetriphosphatase. The enzyme was prepared by the following two methods: *a*) actin-free myosin was prepared following the method described by Szent-Gyorgyi (1); *b*) myosin (containing actin) was prepared according to the method of Greenstein and Edsall (9) with several modifications (10).

Method A Rabbits, after fasting for one to two days, were killed with a blow on the head, were rapidly skinned, eviscerated and were dipped into ice water. After a few minutes the muscles were cut out and were packed in ice. The muscles were then minced in a cooled mincer with holes of 2 mm. in diameter. Every 100 grams of minced muscle were suspended in 150 cc. of ice-cold solution 0.36 M with respect to KCl and 0.18 M with respect to potassium phosphate buffer of pH 6.5 and were extracted for 10 minutes with constant stirring at 0°C. The suspension was then diluted with water of 22°C. temperature, five volumes of water being used for every volume of the KCl-phosphate solution. The solution was rapidly strained through cheesecloth, and the fluid was gently stirred until a flocculent precipitate was formed. The precipitate was separated by high-speed centrifugation at room temperature and the opalescent fluid was diluted with 1.5 volumes of ice-cold water. The water was run in slowly, in about 10 minutes, under constant stirring. The mixture was allowed to stand for one to two hours at 0°C. Afterwards, the supernatant fluid was decanted and the myosin was separated by centrifugation at 0°C. The precipitate was washed by suspending it in 0.02 M KCl and was centrifuged. The precipitate was then dissolved in 0.02 M K_2CO_3 containing 0.01 per cent of phenolphthalein. The carbonate solution was added until the fluid became faint rose in color (pH 8.3). For every gram of myosin 4 cc. of a 2 M KCl solution were added and the suspension, under strong stirring, was diluted with water at 20°C., adding 50 cc. of water for every cc. of KCl solution used. The precipitate was separated by centrifugation and discarded. The supernatant fluid was cooled to 0°C. Afterwards, the fluid was stirred energetically and a solution of one per cent acetic acid was added very slowly until the fluid reached pH 7.0. The precipitate was separated by centrifugation and was dissolved by addition of small amounts of 2 M KCl until the concentration of KCl reached 0.6 M. The mixture was then diluted with 0.6 M KCl until it lost its high viscosity. The solution was stirred energetically and water was run in very slowly until the concentration of KCl was as low as 0.04 M. The precipitated myosin was separated by centrifugation.

Method B All procedures were carried out below 5°C. The muscle of rabbit was frozen with dry ice and ground in a meat grinder to a fine powder. The powdered muscle was extracted with 10 times its volume of a solution 0.5 M with respect to KCl and 0.04 M with respect to $KHCO_3$ (pH 7.8) with stirring for 16 hours. The tissue was removed by high-speed centrifugation. The supernatant fluid was poured into about 15 to 20 times its volume of distilled water and the pH was corrected to 6.8. The suspension was left in the icebox for a few hours. Afterwards, the supernatant fluid was decanted, and to the precipitate separated by centrifugation KCl and $KHCO_3$ were added until the concentration of KCl reached 0.5 M and the concentration of $KHCO_3$ reached 0.05 M. After thorough mixing the suspension was poured into water of 15 to 20 times its volume, the pH was corrected to 6.8 and the mixture was left standing in the icebox for a few hours. Centrifugation, admixture of KCl and $KHCO_3$ and suspension in water were repeated once more. The myosin was then separated by centrifugation and kept in the icebox until used.

Measurement of enzyme activity (*10*). One cc. of enzyme, one cc. of a solution of the sodium salt of ATP and one cc. of the solution of the agents to be tested were mixed and incubated for 30 minutes either at room temperature (23°C.) or at 38°C. To each mixture containing acetylcholine 0.5 mgm. physostigmine salicylate was added. All substances were dissolved in a 0.25 M sodium veronal buffer at pH 8.5. At the end of 30 minutes the reaction was stopped by the addition of 4 cc. of a five per cent trichloracetic acid solution. The mixture was made to 10 cc. with water, was centrifuged at high speed and a suitable aliquot was analyzed for phosphorus. Control experiments were performed with ATP and myosin, with ATP alone, with myosin alone, with ATP and the agents to be tested alone and with myosin and the agents to be tested alone. The alkaloids used, in higher concentrations, interfered with the determination of the phosphorus; therefore, they were used in a concentration of 0.03 mgm. per cc. or less. Because of this limitation, in all experiments the amount of myosin was kept small. The amount of the enzyme that in the control experiments (mixture of ATP and enzyme) split 40 micrograms of phosphorus from a solution containing 3 mgm. of ATP phosphorus in 30 minutes at 38°C. was taken as one enzyme unit.

Calculation. The amount of phosphorus split in the controls containing myosin and ATP was taken as 100 per cent. The amount of phosphorus split in the experimental mixtures was expressed as a percentage of this amount. All results deviating from 100 per cent by more than twice the square root of the sum of the squares of the standard error of mean of the controls and the standard error of mean of the experiments were considered significant

$$(2\sqrt{\text{S.E.}^2\,(\text{control}) + \text{S.E.}^2\,(\text{experiment})}).$$

The Q_P value was calculated following the equation: $\dfrac{\mu\text{g. P} \times 22.4/31 \times 60}{\text{mgm. N} \times 6 \times t(\text{min})}$.

RESULTS

The activity of the enzyme preparation *A* averaged $Q_P = 4000$ (at pH 8.5) and that of preparation *B* averaged $Q_P = 1000$ (at pH 8.5). These activity values were lower than the maximum values given by Szent-Gyorgyi (1) and Bailey (11, 12) probably because in the present experiments maximum purity afforded with the methods used was not achieved. The splitting of phosphate from ATP in the presence of an excess of ATP and decreasing concentrations of the enzyme followed a nearly linear curve. The splitting of phosphorus from ATP by keeping the concentrations of the enzyme constant and increasing the concentration of ATP starting from O followed a nearly linear curve corresponding to the curves found by the collaborators of Szent-Gyorgyi (1) and other investigators (10, 12). The temperature coefficient averaged 1.3 per 10°C., the optimum temperature being 38°C.

The values of the activity of muscle adenosinetriphosphatase in the presence of the agents used are given in table 1. Acetylcholine in low concentrations increased the activity of the enzyme and in concentrations of 0.1 mgm. per enzyme unit the activity of the enzyme was not changed. In higher concentrations, acetylcholine interfered with the determination of phosphorus. The adenosinetriphosphatase activity decreased in the presence of higher concentrations of potassium (see also 10). Acetylcholine increased the activity of the enzyme regardless of the amount of potassium present.

Physostigmine (from 0.5 mgm. per enzyme unit), prostigmine, veratrine, atropine, quinine, ephedrine, morphine, strychnine, caffeine, in concentrations from 0.1 to 0.0001 mgm. per enzyme unit, and d-tubocurarine, in concentrations from 0.01 to 0.0001 mgm. per enzyme unit, did not modify the adenosinetriphosphatase activity. Caffeine and d-tubocurarine, in higher concentrations, decreased the activity of the enzyme.

DISCUSSION

In low concentrations acetylcholine (from 0.01 to 0.0001 mgm. per enzyme unit) slightly increased the activity of muscle adenosinetriphosphatase. These results differ from the observations of Ziff (13). The negative results of Ziff may be attributed to the impurity of the enzyme preparation used and perhaps also to the omission of physostigmine.

D-tubocurarine, in relatively low concentrations (0.1 mgm. per enzyme unit), decreased the activity of adenosinetriphosphatase. The concentrations of the alkaloid required for the depression were higher than those required to modify the effect of acetylcholine and of indirect stimulation in inducing shortening of muscle. They were, however, similar to those that decrease the effect of potassium in inducing shortening on muscle (14) and those required to induce contracture of muscle in vivo (15).

The decreased activity of muscle adenosinetriphosphatase in the presence of higher concentrations of potassium (10) and caffeine may contribute to the effect of these substances in inducing contracture of the muscle (16).

Shortening of muscle may be induced by acetylcholine in vivo and in vitro. The possibility of an interrelationship between the mechanism through which adenosinetriphosphate and acetylcholine induce shortening of muscle is indicated by the observation that subthreshold amounts of adenosinetriphosphate, insufficient by themselves to induce muscle contraction, sensitize the muscle to acetylcholine (17). The physiological importance of acetylcholine may be suggested not only because agents preventing acetylcholine from inducing muscle shortening (e.g., curare) also decrease the effect of indirect stimulation, but because substances decreasing acetylcholine synthesis decrease also the effect of indirect stimulation on the muscle (18). The mechanism through which

TABLE 1. EFFECT OF THE SUBSTANCES ON ACTIVITY OF MUSCLE ADENOSINETRIPHOSPHATASE

SUBSTANCE	NO. OF EXPTS.	MYOSIN PRPN.	AMOUNT OF PHOSPHORUS SPLIT IN PER CENT OF CONTROL FOLLOWED BY THE STANDARD ERROR OF MEAN					
			Amount of substance added (mgm.):					
			10	0.5	0.1	0.01	0.001	0.0001
Acetylcholine	20	A			104 ± 1.5	117 ± 2.6	137 ± 2.2	140 ± 2.9
" and 0.3 M KCl	10	A			103 ± 1.0	114 ± 1.6	139 ± 3.0	137 ± 2.1
Acetylcholine	10	B			103 ± 1.3	110 ± 1.4	139 ± 2.7	134 ± 1.9
" and 0.3 M KCl	10	B			100 ± 0.5	111 ± 0.8	136 ± 2.4	130 ± 1.8
Prostigmine bromide	10	A			106 ± 1.9	101 ± 2.0	105 ± 2.3	102 ± 0.9
	15	B			99 ± 0.7	103 ± 1.1	106 ± 2.1	105 ± 1.0
Atropine	10	A			96 ± 0.6	98 ± 1.0	97 ± 1.1	102 ± 0.4
	10	B			104 ± 0.8	96 ± 0.9	101 ± 0.7	100 ± 1.8
Ephedrine	10	A			101 ± 1.6	107 ± 0.6	106 ± 1.2	110 ± 1.0
	10	B			100 ± 0.7	104 ± 0.5	108 ± 0.9	109 ± 0.4
Morphine	5	A			100 ± 0.8	103 ± 1.0	103 ± 1.3	100 ± 0.7
	5	B			98 ± 1.2	101 ± 1.5	99 ± 0.5	104 ± 1.1
Physostigmine salic.	12	A		99 ± 0.3	100 ± 0.5	103 ± 1.4	104 ± 1.1	101 ± 0.5
	12	B		101 ± 1.0	97 ± 0.8	102 ± 1.0	105 ± 0.4	103 ± 0.4
Quinine	5	A			107 ± 0.9	104 ± 1.1	100 ± 1.1	98 ± 0.8
	5	B			100 ± 0.5	102 ± 0.3	105 ± 1.0	104 ± 1.1
Strychnine	5	A			101 ± 0.4	101 ± 0.5	100 ± 0.3	97 ± 0.5
	5	B			96 ± 0.5	102 ± 0.6	104 ± 0.7	101 ± 0.8
d-Tubocurarine	15	A			73 ± 0.7	98 ± 1.0	105 ± 0.9	103 ± 0.6
	15	B			75 ± 0.5	103 ± 0.5	103 ± 1.0	100 ± 0.8
Veratrine	15	A			109 ± 0.5	104 ± 1.2	105 ± 0.9	106 ± 1.1
	15	B			107 ± 0.9	105 ± 0.7	104 ± 0.4	101 ± 0.2
Caffeine	10	A	68 ± 1.2	88 ± 0.4	97 ± 1.1	103 ± 0.6	99 ± 0.9	105 ± 0.5
	10	B	70 ± 1.5	85 ± 0.6	101 ± 1.0	99 ± 0.3	102 ± 0.4	105 ± 0.7

acetylcholine induces shortening of muscle and improved muscle function is not yet defined. The observations: *a*) that acetylcholine induces shortening of muscle; *b*) that acetylcholine does not induce shortening of actomyosin fibrils in vitro and does not modify the shortening of the fibrils induced by adenosinetriphosphate (5); and *c*) that addition of subthreshold concentrations of adenosinetriphosphate to the extracellular spaces of muscle fibers sensitize the muscle to acetylcholine (17) suggest that acetylcholine induces shortening of muscle by modifying the distribution of adenosinetriphosphate in the muscle cell.

Acetylcholine, by improving the activity of adenosinetriphosphatase, may contribute to a more adequate recovery of the muscle.

Acetylcholine improves the activity of muscle adenosinetriphosphatase independently of the amount of potassium present (table 1). Acetylcholine exerts this action by a mechanism other than substitution for some potassium ion since addition of potassium to the enzyme-substrate system failed to increase the activity of the enzyme and even caused a decrease of the activity of adenosinetriphosphatase. The mechanisms through which acetylcholine and potassium induce shortening of muscle are probably not identical in all details since many substances (adenosinetriphosphate among others, 17) modify the effects of potassium and acetylcholine in inducing muscle shortening in a different manner (14, 17, 19).

SUMMARY

1. The effects of acetylcholine, some alkaloids and caffeine on the activity of muscle adenosinetriphosphatase were investigated.

2. Acetylcholine, in low concentrations, increased the activity of the enzyme.

3. D-tubocurarine, caffeine and potassium, in concentrations corresponding to those inducing muscle contracture, decreased the activity of the enzyme.

4. The other substances used (atropine, ephedrine, morphine, physostigmine, prostigmine, quinine, strychnine and veratrine, in concentrations not interfering with the phosphorus determination) did not modify the activity of the adenosinetriphosphatase.

5. The mechanism of action of acetylcholine on muscle is discussed.

REFERENCES

1. Szent-Gyorgyi, A. Acta Physiol. Scand. **9:** Suppl. 25, 1945.
2. Montigel, C. Helv. Physiol et Pharmacol. Acta **1:** c47, 1943.
3. Guba, F., cited by Szent-Gyorgyi. Page 108.
4. McIntyre, A. R. and I. Braverman. Federation Proc. **6:** 158, 1947.
5. Youngburg, G. F. and M. V. Youngburg. J. Lab. Clin. Med. **16:** 158, 1940; also Kuttner, T. and H. R. Cohen. J. Biol. Chem. **75:** 517, 1927; and Kuttner, T. and L. Lichtensstein. J. Biol. Chem. **86:** 671, 1930.
6. Bock, J. C., and S. R. Benedict. J. Biol. Chem. **20:** 47, 1915 as described in Hawk, P. B., B. L. Oser and W. H. Summerson. Practical Physiological Chemistry, 12th ed. Philadelphia-Toronto: The Blakiston Co., 1947. Pp. 816, 820–821.
7. Needham, D. M. Biochem. J. **36:** 113, 1942.
8. Umbreit, W. W., R. H. Burris and J. F. Stauffer. Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Minneapolis: Burgess Publishing Co., 1945. P. 181.

9. Greenstein, J. P. and J. T. Edsall. J. Biol. Chem. **133:** 397, 1940.
10. Singher, H. O. and A. Meister. J. Biol. Chem. **159:** 491, 1945.
11. Bailey, K. Biochem. J. **36:** 121, 1942.
12. Engelhardt, V. E. Advances in Enzymol. **6:** 147, 1946.
13. Ziff, M. Proc. Soc. Exp. Biol. and Med. **51:** 249, 1942.
14. Torda, C. and H. G. Wolff. J. Lab. Clin. Med. **32:** 1374, 1947.
15. McIntyre, A. R., R. E. King and A. L. Dunn. J. Neurophysiol. **8:** 297, 1945; McIntyre, A. R. and R. E. King. Science **97:** 516, 1943.
16. Gasser, H. Physiol. Revs., **10:** 35, 1930.
17. Torda, C. and H. G. Wolff. This Journal **145:** 419, 1946.
18. Torda, C. and H. G. Wolff. This Journal **146:** 639, 1946.
19. Torda, C. Current Researches Anesthesia & Analgesia **23:** 74, 1944; Proc. Soc. Exp. Biol. and Med. **51:** 121, 1943; Torda, C. and H. G. Wolff. Proc. Soc. Exp. Biol. and Med. **56:** 89, 1944; **57:** 236, 1944; **58:** 29, 1945; This Journal **145:** 608, 1946; **146:** 567, 1946: J. Lab. Clin. Med. **31:** 650, 1946; J. Exp. Med. and Surg. **4:** 50, 1946.

RENAL EXCRETION AND VOLUME DISTRIBUTION OF SOME POLYETHYLENE GLYCOLS IN THE DOG

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The polyethylene glycols (polyglycols, polyoxyethylene glycols) are long chain, highly polymerized hydrocarbon compounds characterized by regularly spaced ether linkages and, presumably, terminal hydroxyl groups. The degree of polymerization can be controlled in their preparation so that products of any consistency from viscous liquids to pasty or solid materials can be formed. The products available commercially are mixtures of various molecular weights, the average of which is designated by a number appended to the trade-mark name. In the present report polyethylene glycols ranging in molecular weight from 400 to 6000 have been investigated and these will be referred to as PEG-400, PEG-1000, PEG-1540, PEG-4000 and PEG-6000.[1]

The observations recorded herein are the outgrowth of an extended toxicological study of these compounds. It has been shown that, injected intravenously, the polyglycols are rapidly and quantitatively excreted in the urine (1). A further investigation of their renal clearance rate has revealed that, with one exception, this rate is identical with that of creatinine in the dog, thereby placing these polyglycols among that class of substances which are excreted by glomerular filtration without tubular participation. Because of the supplementary evidence which additional substances of this nature afford to the filtration-resorption theory of kidney function, the following results are presented.

METHODS

Analytical procedures. Creatinine determinations were done by the Folin-Wu method (2) on zinc hydroxide (3) filtrates of plasma and appropriate dilutions (urine to plasma ratio of about one) of urine. With the plasma levels of creatinine attained with the present technique, a 1:50 dilution was required in order to bring the readings of samples within the optimum range of the photoelectric colorimeter.

All the polyglycols were determined in both plasma and urine by a modification of a gravimetric procedure published earlier (4) involving their precipitation from an acid solution by silicotungstate in the presence of barium. In the analysis of plasma, 5 ml. of plasma is diluted to 40 ml. with water, and the proteins are precipitated by the addition of 10 ml. of 20 per cent trichloracetic acid. After removal of the coagulum by centrifugation, 40 ml. of clear filtrate is diluted to about 200 ml., and 5 ml. of 10 per cent barium chloride solution is added. Any turbidity that should appear at this point as a result of the presence of sulfate may be removed by filtering the sample quantitatively through Whatman No. 42 or similar paper. Afterward the polyglycol is precipitated in the cold by the addition of 5 ml. of 10 per cent silicotungstic acid solution, and the remainder of the analysis completed according to the original method.

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[1] These were obtained from Carbide and Carbon Chemicals Corporation under the names of polyethylene glycol 400, and 'carbowax' compounds 1000, 1540, 4000, and 6000, respectively. The name 'carbowax' is a registered U. S. trade-mark.

In the analysis of urine, an aliquot of the urine collection calculated to contain about 20 to 30 milligrams of polyglycol is diluted to about 200 ml.; it is then acidified with 10 ml. of 20 per cent trichloracetic acid, and barium chloride and silicotungstic acid are added as described. The sample is generally so dilute that turbidity seldom appears after addition of the barium.

For purposes of maximum accuracy in calculating the quantity of polyglycol represented by the amount of residue weighed, use has been made of equations derived by the method of least squares[2] from data obtained by carrying replicate samples of known amounts of each of the polyglycols in the range of 5 to 50 milligrams through all the steps of the analytical procedure.

Judging from the recovery of known amounts added to blank samples of dog plasma, each of the polyglycols from 400 to 4000 may thus be determined in plasma in the range of 100 to 500 mgm. per cent with an average error not exceeding two per cent. In the case of PEG-6000, however, recoveries averaged about eight per cent low. As this experience with PEG-6000 is the single exception, and as the error in its plasma determination is not in the direction to alter the eventual conclusion, the results of the experiments involving it are included.

Clearance technique. Because of the relative lack of sensitivity of the analytical method as applied to blood, it is desirable to work with plasma concentrations of polyglycol of the order of 100 mgm. per cent and higher. As these compounds are but very slightly absorbed from the gastro-intestinal tract (1), and very slowly from subcutaneous depots, it was necessary to resort to constant intravenous infusion in order to maintain plasma levels of this degree. For this purpose, a pump was used consisting of a 100 ml. glass syringe, the plunger of which was advanced by a screw driven by a low-speed synchronous motor. The rate of delivery under these conditions was 2.4 ml. per minute. The plasma concentration of polyglycol obtainable was varied by altering the concentration in the saline infusion fluid in the range of 2 to 5 per cent. It was found convenient to include the reference substance, creatinine, in the infusion fluid in a concentration of 0.5 per cent. At the start of the infusion, a priming dose consisting of 4 grams of polyglycol and one gram of creatinine in 20 ml. of saline was injected into the infusion tubing while the pump was in operation.

All experiments were performed on female dogs, with prior hydration, under light pentobarbital anesthesia. The animal was placed in the supine position on a padded board, the bladder was catheterized and the infusion was commenced via the jugular vein. The infusion was allowed to proceed for at least an hour before the first clearance period in order to insure thorough equilibrium. All periods were 15 minutes in length, with the blood sample being drawn at the midpoint from either the saphenous vein or the opposite jugular. The bladder was rinsed with saline at the beginning and again at the end of each period. When it became necessary to interrupt the infusion in order to refill the syringe, a short length of time was allowed for re-equilibration, although the operation of filling could usually be accomplished in less than 30 seconds.

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[2] The authors are indebted to Carrol S. Weil of this Fellowship for all statistical analyses in this work.

TABLE 1. SIMULTANEOUS RENAL CLEARANCES OF CREATININE AND POLYGLYCOLS IN ANESTHETIZED DOGS OVER 15-MINUTE PERIODS

DOG	EXPERIMENT	NO. OF PERIODS	POLYGLYCOL PLASMA RANGE	AVERAGE POLYGLYCOL CLEARANCE	POLYGLYCOL CLEARANCE / CREATININE CLEARANCE	
					Range	Average
				PEG-400		
			mgm. per cent	ml/min.		
002	1	4	214–236	43	1.02–1.08	1.06
429	1	2	223–231	38	1.01	1.01
155	1	4	198–215	44	0.92–1.10	1.00
729	1	3	181–189	39	1.04–1.07	1.05
002	2	4	207–219	41	0.92–0.98	0.94
			PEG-1000			
062	1	4	246–270	44	0.94–1.01	0.98
155	2	4	112–138	52	0.97–1.03	0.99
157	1	4	268–280	40	0.89–1.01	0.94
062	2	4	268–281	41	0.87–0.98	0.92
			PEG-1540			
155	3	4	233–251	42	0.96–1.00	0.98
729	2	4	210–223	52	0.94–1.00	0.98
157	2	4	105–114	53	0.99–1.05	1.02
			PEG-4000			
062	3	2	153–160	39	0.97–1.00	0.99
665	1	4	194–206	52	1.05–1.10	1.06
663	1	4	282–290	39	0.93–0.95	0.94
729	3	4	122–154	52	0.91–1.03	0.98

RESULTS

Clearance ratios. A total of 59 periods were obtained in 16 experiments (table 1) on PEG-400, PEG-1000, PEG-1540 and PEG-4000. Considering these 59 periods as one group, which is justifiable in view of the small dispersion of the results about the mean, the average ratio of the polyglycol clearance to the simultaneous creatinine clearance is 0.99 with a standard deviation of 0.06. All the values fall within the limits of two standard deviations, and 95 per cent of them are between 0.90 and 1.10. These results therefore justify the conclusion that in the dog these polyglycols are excreted by a mechanism identical with that of creatinine excretion.

Eight experiments with PEG-6000 are recorded in table 2. The results show that the clearance of this polyglycol is consistently and significantly less than that of the reference substance, a circumstance that is generally taken to indicate tubular resorption of the former. Neither saturation of tubular excretory activity with diodrast nor saturation of tubular resorptive activity with glucose produced any significant alteration in the simultaneous polyglycol-creatinine clearance ratio. This lack of effect of glucose demonstrates that such tubular resorption of PEG-6000 as does take place is of the 'passive' variety, or at least not competitive with the mechanism for glucose. The concept of tubular resorption of PEG-6000 based on diffusibility alone is not attractive, however, because from all other considerations, e.g., volume distribution, it is the least diffusible of the series, as might be expected from its greater molecular weight.

TABLE 2. SIMULTANEOUS RENAL CLEARANCES OF CREATININE AND PEG-6000 IN ANESTHETIZED DOGS OVER 15-MINUTE PERIODS

DOG	EXPERIMENT	NO. OF PERIODS	POLYGLYCOL PLASMA RANGE	AVERAGE POLYGLYCOL CLEARANCE	POLYGLYCOL CLEARANCE / CREATININE CLEARANCE	
					Range	Average
			mgm. per cent	*ml/min.*		
062	4	4	354–377	28	0.69–0.73	0.70
002	3	4	339–357	27	0.73–0.83	0.77
157	3	4	261–301	40	0.68–0.79	0.75
155	4	4	281–293	33	0.65–0.71	0.68
157	4[1]	3	68–212[2]	34	0.76–0.87	0.83
155	5[1]	4	79–227[2]	32	0.76–0.85	0.79
665	2[1]	4	90–247[3]	31	0.56–0.72	0.65
729	4[1]	4	146–388[3]	20	0.49–0.64	0.60

[1] These last four sets of clearances were performed following a single intravenous injection of about 10 grams of the polyglycol combined with 5 grams of creatinine. One-half hour was allowed for equilibration, after which simultaneous blood and urine samples were taken at consecutive 15-minute intervals. Intermediate blood concentrations were calculated according to Newman *et al.* (5).

[2] Tubular excretory activity saturated by means of a constant intravenous infusion of diodrast.

[3] Tubular resorptive activity saturated by means of a constant intravenous infusion of glucose.

Volume distribution of the polyglycols. In conjunction with determinations of the renal clearance rate, it seemed desirable to get some data on the volume distribution of the polyglycols. These facts were obtained by following the cumulative urinary excretion after single intravenous injections of known amounts of each member of the series. The total amount recovered in the urine up to any given time was deducted from the amount injected, and the difference divided by the plasma concentration (in mgm. per liter) determined at that time. The results, which are presented in table 3, are consistent with the interpretation that the polyglycols diffuse into a volume approximating that of the extra-cellular fluid of the body. In none of these calculations has allowance been made for the fact that the lower molecular weight polyglycols do undergo some limited destruction in the body. The extent of this destruction has not been ascertained in dogs, but in the work noted earlier (1) it was found that, whereas 96 per cent PEG-6000 could be recovered in the urine during the 12 hours following a single intravenous injection in humans, only an average of 85 per cent of PEG-1000 could be accounted for in this manner. It would be logical to expect, therefore, that a lower molecular weight compound such as PEG-400 would be metabolized to a somewhat greater extent: This destruction

TABLE 3. VOLUME DISTRIBUTION OF POLYGLYCOLS IN ANESTHETIZED DOGS

DOG	WEIGHT	AMOUNT INJECTED	TIME FROM BEGINNING OF INJECTION	CUMULATIVE RECOVERY IN URINE	PLASMA CONCENTRATION	POLYGLYCOL SPACE	
						Volume	% Weight
				PEG-400			
	kgm.	*mgm.*	*min.*	*mgm.*	*mgm/l.*	*l.*	
002	10.8	13,352	30	2383	3155	3.48	32.2
			60	2680	2280	4.68	43.3
			75	2888	2393	4.37	40.5
				PEG-1000			
665	11.3	15,481	30	6515	3241	2.77	24.5
			60	9116	1937	3.29	29.1
			75	10,084	1580	3.41	30.2
				PEG-1540			
729	13.4	9316	30	4479	1864	2.59	19.3
			60	6276	952	3.20	23.9
663	10.6	14,272	30	4434	4736	2.08	19.6
			60	7204	2489	2.84	26.8
			75	8208	1921	3.16	29.8
				PEG-4000			
155	14.5	15,052	30	7104	3377	2.35	16.2
			60	9884	1695	3.05	21.0
			75	10,787	1284	3.32	22.9
				PEG-6000			
157	14.5	15,025	30	8090	4015	1.73	11.9
			60	9843	1607	3.22	22.2
			75	10,347	1225	3.82	26.3
665	10.9	15,025	30	8015	3938	1.78	16.3
			60	11,088	1416	2.78	25.5
			75	12,094	927	3.16	29.0

is undoubtedly the explanation of the high values for volume distribution given by this substance.

DISCUSSION

In the absence of a simple, highly sensitive, and sufficiently accurate analytical method for their estimation in plasma, the use of the polyglycols in the determination of glomerular filtration rate has nothing to recommend it over the employment of a number of other substances available for this purpose. The present results are reported simply as a matter of theoretical interest in the field of renal physiology. Should such a method be found, however, the polyglycols should have all the advantages possessed by any of the other materials, including chemical stability, lack of buffer action and low toxicity. In none of the experiments set forth have any evidences of acute toxicity of these compounds been seen, even after the administration of as much as 15 grams in a single injection. Several of the animals have been in intermittent use during a period of more than a year in experiments of this type, with no apparent evidences of chronic toxicity. Several papers have appeared previously on the pharmacological action of these compounds (6, 7, 8).

PEG-400 and PEG-1000 may be filtered quantitatively through cellophane (duPont No. 300 PT) from both plasma and saline solutions, but the percentage filterable diminished in the case of PEG-1540 and fell off sharply with PEG-4000 and PEG-6000. Since even less of the last two can be filtered from saline than from plasma, the permeability of the membrane rather than protein-binding is the factor responsible. Control experiments showed this type of membrane to be relatively impermeable to inulin.

No explanation is offered of the anomalous behavior of PEG-6000 as regards its apparent resorption from the glomerular filtrate. This circumstance is particularly unexpected in view of the fact that a major portion of this polyglycol is very probably composed of molecules that are present in the lower molecular weight compounds.

SUMMARY

The renal clearance rates of a series of polyethylene glycols ranging in molecular weight from 400 to 6000 have been determined in the dog.

Polyethylene glycols of average molecular weights of 400, 1000, 1540 and 4000 are cleared from the plasma at a rate identical with that of creatinine in the normal, lightly-anesthetized animal.

The ratio of the clearance of a polyethylene glycol of average molecular weight 6000 to that of creatinine determined simultaneously is consistently and significantly less than one. This ratio is essentially unaffected by extreme hyperglycemia, or by the intravenous infusion of diodrast in an amount sufficient to saturate tubular excretory activity.

Following intravenous injection, all these polyglycols appear to be distributed in the extracellular fluid of the body. The polyglycols of lower molecular weight undergo a limited amount of destruction.

REFERENCES

1. Shaffer, C. B. and F. H. Critchfield. J. Am. Pharm. Assoc., Sci. Ed. **36:** 152, 1947.
2. Folin, O. and H. Wu. J. Biol. Chem. **38:** 81, 1919.
3. Somogyi, M. J. Biol. Chem. **86:** 655, 1930.
4. Shaffer, C. B. and F. H. Critchfield. Anal. Chem. **19:** 32, 1947.
5. Newman, E. V., J. Bordley and J. Winternitz. Bull. Johns Hopkins Hosp. **75:** 253, 1944.
6. Smyth, H. F., Jr., C. P. Carpenter, C. B. Shaffer, J. Seaton and L. Fischer. J. Ind. Hyg. Toxicol. **24:** 281, 1942.
7. Smyth, H. F., Jr., C. P. Carpenter and C. B. Shaffer. J. Am. Pharm. Assoc., Sci. Ed. **34:** 172, 1945.
8. Smyth, H. F., Jr., C. P. Carpenter and C. B. Shaffer. J. Am. Pharm. Assoc., Sci. Ed. **36:** 157, 1947.

INFLUENCE OF THYROID STATUS ON THE ELECTROCARDIOGRAM AND CERTAIN BLOOD CONSTITUENTS OF THE SHEEP[1]

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The recent development of methods for the formation on a large scale of thyroactive iodinated proteins (see review by Reineke, 1) together with the discovery of the thyroid-inhibitory properties of compounds containing the thiourylene radical (2–4) has made possible many investigations on the influence of the thyroid secretion status on the productive processes of domestic animals, as reviewed by Blaxter (5). In view of the impending application of these compounds in several fields of animal production it seemed of interest to investigate the influence of varying levels of thyroid activity on some of the physiologic functions of the larger domestic animals.

The present paper is confined to data on the effect of the thyroid status on the EKG and blood composition of the domestic sheep.

METHODS AND PROCEDURE

Nine sheep approximately eight months of age and including 6 females and 3 castrate males were used. Throughout the experiments the animals were fed a mixed legume hay supplemented with whole oats and cottonseed meal.

After a preliminary period of two weeks in which control observations were taken on all animals, 5 of the sheep were thyroidectomized and 4 were retained as normal controls. After being maintained under these conditions for three months the thyroidectomized animals were given synthetic thyroprotein[3] orally at the rate of one gram daily per 45 kgm. body weight during a period of three weeks. The dosage of thyroprotein was then doubled during a final two-week period. At the same time the intact controls were given thiouracil[4] at the rate of 0.2 gram per kgm. body weight daily in order to compare the effects of thiouracil administration with the previously observed effects of thyroidectomy.

The method of recording electrocardiograms was essentially the same as that used by Alfredson and Sykes (6) in cattle. The only deviation from this technique was that the area for application of the electrodes was first wiped thoroughly with ether before the application of the electrode paste. The apparatus employed was a Hindle No. 2 string galvanometer.

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[1] Published with the approval of the Director of the Agricultural Experiment Station as Journal Article No. 877 (n.s.).

[2] Government of India Scholar, on leave from the Imperial Veterinary Research Institute, Izatnagar, U. P. India.

[3] We are indebted to Dr. W. R. Graham, Jr., Cerophyl Laboratories, Inc., 2438 Broadway, Kansas City, Mo., for a supply of 'Protamone', the synthetic thyroprotein used in these trials.

[4] Kindly supplied by Dr. Stanton Hardy, Lederle Laboratories, Pearl River, New York.

Records were taken on all animals at weekly intervals. Three[5] normal records were obtained on the groups to be thyroidectomized and 11 on the groups which received thiouracil. All records were taken in the afternoon.

Blood and serum analyses were made at intervals of two weeks during the first three-month period and weekly thereafter. Hemoglobin was determined as alkaline hematin and computed from a standard curve established by the oxygen capacity method. Blood glucose was determined by the method of Somogyi (7). Fat was determined by the volumetric method of Allen (8) which includes the neutral fat and cholesterol, but not the phospholipids. Serum was analyzed for calcium by the Clark and Collip method (9), magnesium and inorganic phosphorus by the method of Fiske and Subbarow (10) and protein from the specific gravity (11). Body weights were recorded weekly.

TABLE 1. PARTIAL STATISTICAL SUMMARY OF EKG VALUES TAKEN FROM LEAD II OF ALL THE NORMAL RECORDS IN NINE SHEEP

	MIN.	MAX.	MEAN	STANDARD DEVIATION
Heart rate (per min.)	88	142	112	15.2
Intervals, *seconds*				
PR	0.08	0.14	0.102	0.001
QRS	0.02	0.04	0.038	0.017
QT	0.22	0.32	0.271	0.03
K (Bazett)	0.30	0.42	0.367	0.03
Potentials, *millivolts*				
P	0.05	0.20	0.103	0.03
Q	0.10	0.30	0.169	0.08
R	0.10	0.40	0.171	0.09
S	0.10	0.10	0.10	—
T	0.10	0.60	0.284	0.11

RESULTS AND DISCUSSIONS

Normal EKG records. A summary of the various EKG values from the normal records (table 1) shows that the duration of the intervals is somewhat shorter and the potential of the deflections is much smaller than those reported in dairy cattle (6). The form of the deflections, however, is quite similar. The shorter interval length may be due in part to the more rapid heart rate and in part to the smaller size of the heart as compared to cattle. The smaller potential of the waves could be due to a higher skin resistance in sheep or to a less favorable cardiac electrical axis with respect to the leads than in cattle. Because of the small number of animals used these factors could not be checked satisfactorily in the present study.

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[5] This excludes *animal No. 70* on which only two normal records were obtained.

Variations between serial electrocardiograms occurred in all but one individual. The changes were limited in four animals to some inconstant variation in the direction of the potential in T_3. In one sheep T_2 was upright in one record and inverted in all of the other normal tracings. Another showed some variation in potential of T_2 and slight variations in the form of QRS_2, while QRS_3 of the second normal record was different in appearance from all the other records. One animal showed some variations in the form of QRS_3 and T_3, while QRS_2 was upright in the first normal record and diphasic in the balance of the normal tracings.

TABLE 2. COMPARISON OF NORMAL EKG VALUES WITH THOSE OBTAINED FOLLOWING THYROIDECTOMY, REPLACEMENT THERAPY, AND THIOURACIL ADMINISTRATION

GROUP NO.	PROCEDURE	NO. ANIMALS	HEART RATE (PER MIN.)	INTERVALS			K (BAZETT)	POTENTIALS				
				PR	QRS	QT		P	Q	R	S	T
				sec.	*sec.*	*sec.*		*mv.*	*mv.*	*mv.*	*mv.*	*mv.*
	Av. of all normal records	9	112	0.10	0.038	0.271	0.37	0.10	0.169	0.171	0.10	0.284
I	90 days after thyroidectomy	5	72	0.11	0.035	0.295	0.32	0.10[1]	0.100[1]	0.150	—	0.125
	After 21 days on thyroprotein (1 gram/45 kgm.)	5	87	0.10	0.035	0.285	0.34	0.12[1]	0.133[1]	0.175	—	0.325
	After an additional 14 days on thyroprotein (2 grams/45 kgm.)	5	133	0.09	0.032	0.235	0.35	0.10	0.175	0.140	—	0.275
II	After 21 days on thiouracil (0.2 gram/kgm.	4	94	0.10	0.042	0.260	0.33	0.11	0.350	0.075[2]	0.10[1]	0.225
	After 35 days on thiouracil (0.2 gram/kgm.)	3	91	0.10	0.027	0.300	0.37	0.08	0.360	—	—	0.416

[1] One animal. [2] Two animals.

These observations may serve to emphasize the necessity for recording several serial normal tracings when obtaining EKG records by means of the three standard Einthoven leads in experiments involving sheep. This has also been shown to be true in cattle (6).

EKG following thyroidectomy. The outstanding deviations from the normal (table 2) following thyroidectomy were a decrease in heart rate and a decrease in potential of the *T* wave. The changes in rate became evident at periods varying from 6 to 12 weeks following thyroidectomy. The decrease in potential of the *T* wave appeared to occur later than the decrease in rate. It is possible that the difficulty in measuring waves initially so small may have contributed to this discrepancy. Thyroprotein administration at the 1 gram level resulted in restoration of the normal amplitude of the *T* wave and an increase in the heart rate approaching the control value. At twice this dosage of thyroprotein there was no further change in potential but a considerable increase in the heart rate to an average of 133 beats per minute as compared to the control value of 112 per minute. Table 2 shows no significant differences in the potentials of the other waves or in the lengths of the various intervals as a consequence of thyroidectomy and subsequent feeding of thyroprotein. Due to the unfavorable nature of the electrocardiograms obtained by the three standard leads in sheep, however, it was not possible to measure the potentials of the deflections, other than *T*, with sufficient accuracy definitely to preclude any minor changes in the size of these waves.

EKG results following thiouracil. The decrease in heart rate (table 2) was much smaller and more variable than that following thyroidectomy and was evident at the end of the first week of thiouracil feeding. In view of the small number of animals used, this change is perhaps less significant than that which occurred following thyroidectomy. Contrary to what might be expected from the known action of thiouracil upon thyroid function, the decreased rate was accompanied by a marked increase in potential of the *T* wave. From inspection of the records of the thyroidectomized group it was noted that while the decrease in heart rate following thyroidectomy was evident earlier than the decrease in potential of the *T* wave, at no time was there an increase in potential coincident with this decreased rate as was evident following the administration of thiouracil. This may indicate pharmacologic actions of this drug other than its inhibitory influence on the thyroid.

Blood composition. The only significant change in blood composition that can be related to changes in thyroid status was in the blood fat. In clinical investigations it has been observed (12) that the blood cholesterol level increases markedly in hypothyroid individuals, but remains at the normal level or only slightly below in hyperthyroidism (13).

In the present investigation the response to thyroidectomy (table 3) appears to be atypical in that the thyroidectomized sheep actually have a lower blood fat level than the normal controls. However, thyroprotein therapy resulted in a slight depression of the blood fat level of this group from 118 down to 113 mgm. per cent. Administration of thiouracil to the normal group resulted in a highly significant increase in the blood fat level from the normal of 136 mgm. per cent up to 194 mgm. per cent. A similar rise in blood cholesterol during thiouracil administration has been reported by Barrick *et al.* (14).

It is well established that the blood sugar level can be increased by stimulation with thyroidal substances although the extent of the rise appears to depend upon the level of hyperthyroidism obtained. In the present instance the blood sugar levels during thyroidectomy and during treatment with thiouracil did not differ appreciably from the control value. During treatment with thyroprotein there was a small, but statistically insignificant rise above the normal. No changes in the level of blood hemoglobin, serum protein, calcium, inorganic phosphorus or magnesium that could be related to the thyroid status were observed.

The failure to observe any alteration in the level of mineral constituents in the blood is perhaps worthy of comment inasmuch as it has been observed that calcium excretion is increased markedly in toxic goiter (15). Slight hyperthyroidism, on the other hand, has been reported to increase calcium retention in growing children (16). In the absence of calcium balance data in the present investigations it cannot be ascertained whether or not any changes in mineral excretion or retention were effected by the alterations in thyroid status. In recent investigations Blaxter (17) observed that when thyroprotein was given to sheep in considerably higher dosages than were employed here extreme depletion of calcium and phosphorus occurred.

TABLE 3. EFFECT OF THYROIDECTOMY, THIOURACIL AND THYROPROTEIN ADMINISTRATION ON CERTAIN BLOOD CONSTITUENTS OF SHEEP

	BLOOD		BLOOD SERUM					BODY WT.	REMARKS
	Hb	Sugar	Protein	Fat	Ca.	Inorg. P.	Mg.		
	grams %	*mgm.* %	*grams* %	*mgm.* %	*mgm.* %	*mgm.* %	*mgm.* %	*kgm.*	
Mean	9.8	52.0	7.62	136.3	10.7	7.3	2.58	33.8	Normal control 15 weeks
Standard deviation	0.44	9.2	1.39	17.1	6.03	0.92	0.57	3.80	
Mean	10.1	50.3	8.17	117.8	11.4	7.4	2.58	40.5	Thyroidectomy 13 weeks
Standard deviation	0.51	8.16	1.11	11.16	6.63	0.78	0.37	4.47	
Mean	9.81	55.8	8.30	113.0				46.4	Thyroprotein daily 1 gm./45 kgm. body wt., 3 weeks
Standard deviation	2.43	13.8	2.06	26.4				14	
Mean	9.81	57.3	7.28	102.2				44.1	Thyroprotein daily 2 gm./45 kgm. body wt., 2 weeks
Standard deviation	3.00	14.0	2.48	31.0				13.4	
Mean	9.0	54.6	7.4	194.1				36.1	Thiouracil daily 0.2 gm./kgm. body wt., 5 weeks
Standard deviation	1.83	11.50	1.63	39.50				8.25	

The number of analyses averaged for each blood constituent in the respective groups is as follows: normal control, 27; thyroidectomy, 34; thyroprotein, 1 gram dosage, 14; 2 grams dosage, 9; thiouracil, 18.

The average initial body weights before operation of the thyroidectomized and normal control sheep were 34 and 31 kgm., respectively. Ninety days after thyroidectomy the thyroidectomized animals averaged 40.5 kgm. compared to 33.8 kgm. for the controls. Statistical analysis showed the difference in rate of gain to be highly significant, although nothing is known about the composition of the gains. During the period of treatment with thyroprotein and thiouracil no significant changes in body weight occurred. This is good evidence that the dosages of thyroprotein employed, although sufficient to produce definite effects on the heart action and blood fat level as described earlier, were well within the range of physiological tolerance of sheep.

SUMMARY

The thyroid status of domestic sheep was modified experimentally by means of thyroidectomy followed by thyroprotein therapy and also by the administration of thiouracil. Under each of the experimental conditions data were obtained on the EKG and certain constituents of the blood.

In the EKG studies the outstanding deviations from the normal following thyroidectomy were a decrease in heart rate and a decrease in potential of the *T* wave. Thyroprotein administration at the rate of 1 and 2 grams per 45 kgm. body weight restored the EKG pattern to normal or above. Thiouracil administration resulted in an atypical increase in potential of the *T* wave in addition to some reduction in heart rate.

The 'blood fat' as determined on serum by the method of Allen did not show the expected rise following thyroidectomy. The blood fat level declined slightly, however, after treatment with thyroprotein. Thiouracil administration resulted in a sharp and highly significant increase in the blood fat levels. No appreciable alterations in the blood sugar level after thyroidectomy or treatment with thiouracil were noted. There was a small, but statistically insignificant, rise in the blood sugar when thyroprotein was fed.

No deviations that could be related to the thyroid status were observed in the blood hemoglobin content or the serum protein, calcium, phosphorus or magnesium.

The authors wish to express their sincere appreciation to Professor George A. Brown, Head, Department of Animal Husbandry, Michigan State College, for supplying the sheep used in these investigations.

REFERENCES

1. Reineke, E. P. Vitamins and Hormones. Academic Press, Inc., New York. **4**: 207, 1946.
2. Richter, C. P. and K. H. Clisby. Arch. Path. **33**: 46, 1942.
3. Mackenzie, C. G. and J. B. Mackenzie. Endocrinology **32**: 185, 1943.
4. Astwood, E. B. J. Pharmacol. and Exp. Therap. **78**: 79, 1943.
5. Blaxter, K. L. J. Animal Sci. In press, 1947.
6. Alfredson, B. V. and J. F. Sykes. J. Agr. Research **65**: 61, 1942.
7. Somogyi, J. J. Biol. Chem. **86**: 655, 1930.
8. Allen, N. N., Minn. Agr. Expt. Sta. Tech. Bull. **130**, 1938.
9. Clark, E. P. and J. B. Collip. J. Biol. Chem. **63**: 461, 1925.
10. Fiske, C. H. and Subbarow, Y. J. Biol. Chem. **66**: 375, 1925.
11. Weech, H. A., E. B. Reeves and E. Goettsch. J. Biol. Chem. **113**: 167, 1936.
12. Hurxthal, L. M. and H. M. Hunt. Ann. Int. Med. **9**: 717, 1935.
13. Schmidt, L. H. and H. B. Hughes. Endocrinology **22**: 474, 1938.
14. Barrick, E. R., W. M. Beeson and F. N. Andrews. J. Animal Sci. **5**: 416, 1946.
15. Aub, J. C., C. Heath and M. Ropes. J. Clin. Invest. **7**: 97, 1929.
16. Johnston, J. A. and J. W. Maroney. Am. J. Diseases Children **58**: 1168, 1938.
17. Blaxter, K. L. J. Agr. Sci. In press, 1947.

MEASUREMENT OF ALVEOLAR PRESSURE IN HUMAN SUBJECTS[1]

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The resistance offered by the respiratory tract to air flow is an important factor in the work of breathing. However, there are relatively few data in the literature giving the magnitude of this resistance. Rohrer (1) gives values for the respiratory tract as a whole and for its various subdivisions. His values are derived theoretically by application of physical laws for the flow of gas in tubes to measurements of respiratory tracts obtained from cadavers.

To evaluate the resistance experimentally one needs to know simultaneously the rate of flow of air in the respiratory tract and the pressure gradient that exists between the alveoli and the outside. Although the former value is rather easily obtained, the latter is not. The most direct method of obtaining the pressure in the alveoli would be to insert a cannula into an alveolus. This method has been applied to dogs by Vuilleumier (2), but is obviously not suited to humans.

Another approach is the measurement of the intrapleural pressure by the method of Neergaard and Wirz (3). The difference between the static and dynamic intrapleural pressures represents the alveolar pressure plus the pressure required to overcome the viscous resistance of the lung tissue. The latter factor cannot be readily evaluated and the work of Bayliss and Robertson (4) on cat lungs indicates that it is not a negligible quantity. Vuilleumier (2) estimates that it amounts to about 20 per cent of the alveolar pressure.

A more indirect method, but one which readily lends itself to human subjects, is one proposed by Neergaard and Wirz (3) and more recently elaborated by Vuilleumier (2). Neither of these authors gives any extensive data, however. It is the purpose of the present paper to call attention to this interesting method and to present some data obtained from its application. The principle is as follows: The subject breathes in and out through a pneumotachograph which records the volume rate of flow on moving paper by way of a differential optical manometer. Another manometer records the pressure at the mouth piece. If the distal end of the system be suddenly occluded at any time during the breathing cycle, the flow drops to zero and the pressure in the whole system becomes equalized, resulting in a sudden rise or fall of pressure at the mouth, depending on whether the interruption occurred during inspiration or expiration.

This sudden change of pressure at the mouth is taken to be a measure of the pressure gradient from alveoli to mouth that existed at the moment of interruption. The justification for this is as follows: The system at any given moment of the respiratory cycle can be regarded as being made up of numerous volumes of air each at its own pressure. When the flow is occluded and the pressure equalized, the equalized pressure multiplied by the total volume is the sum of all the initial pressure-volume products. If the volume of the alveoli is large compared to the other volumes (dead space of subject and apparatus), then the equalized pressure is essentially equal to the pressure that existed in the alveoli at the moment of interruption.

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[1] Work done under contract with Air Materiel Command, Wright Field. A preliminary report appeared in Fed. Proc. 6: 173, 1947.

[2] Visitor in this laboratory from The Johns Hopkins Hospital, Baltimore, Maryland.

EXPERIMENTAL

The tachograph[3] used in the experiments employs a sintered metal (Porex) disc three inches in diameter as a resistance. The pressure drop across this had a linear variation with rate of flow of about $\frac{0.3 \text{ cm.H}_2\text{O}}{100 \text{ cc/sec.}}$. The differential pressure of the tachograph and the pressure at the mouth were in most experiments measured by use of rubber membrane optical manometers, but in later experiments a Statham strain gage pressure transmitter feeding into a string galvanometer was used for one or both pressures.

Two types of interrupting devices were used. One was a metal stopcock placed in the system distal to the pneumotachograph. The handle of the cock was equipped with crank so that it could be turned easily by hand at a rate of two or three rotations a second. The bore of the cock was so constructed that the cock was open about one third of the time and closed about two thirds of the time.

Another apparatus for producing sudden interruptions was a stiff flat spring (made from a 100/sec. tuning fork) with one end securely fixed and with a rubber stopper larger in diameter than the distal end of the tachograph attached near the free end. The free end of the spring was held poised by the rim of a brass wheel so that the stopper was normally well away from the opening of the tachograph and offered no resistance to air flow. A notch was cut in the rim of the wheel at one point so that when the wheel was rotated the spring was suddenly released and the tachograph occluded once each revolution. The time required for interruption was less than 0.01 seconds. The duration of the interruption was one fifth of the time for a complete revolution of the wheel. The shaft of the wheel was equipped with a crank so that it could be turned manually at any desired speed.

Determination of relationship between rate of air flow and alveolar pressure. The subject sat connected to the apparatus by a rubber mouth piece and with the interrupter wide open. After a brief period to allow the subject to become

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[3] This instrument was built according to the model described in Memorandum Report Serial No. TSEAA-696-107A from the Air Materiel Command, Wright Field, prepared by Wayland E. Hull, April 28, 1947.

We wish to thank Dr. W. O. Fenn for originally suggesting this problem to us and for his advice during the course of the investigation.

accustomed to the apparatus, the valve was operated so as to interrupt the flow several times during each breathing cycle and a record covering several cycles was taken. The subject was then requested to breathe at rates of flow somewhat higher than normal and another record was taken. A sample of the sort of record obtained is shown in figure 1.

This record shows that at each interruption the rate of flow suddenly drops (or rises) to zero, where it remains during the period of interruption. At the same time the pressure at the mouth abruptly increases (or decreases) and then performs a series of damped oscillations around a slowly rising (or falling) line. The height above the line of zero pressure at which a line drawn through the centers of oscillations intersects the initial abrupt rise in pressure is taken as representing the pressure in the lungs at the moment of interruption. The pressure gradient between mouth and alveoli is obtained by subtracting the pressure in the mouth at the onset of interruption. The initial overshoot and subsequent damped oscillations are believed to represent the conversion of kinetic energy to pressure energy and its subsequent dissipation as pressure waves, which are reflected back and forth in the airways (a sort of 'water hammer' effect). The fact that the center of oscillation of these waves continues to rise (or fall) indicates that the chest continues to move during the period of interruption.

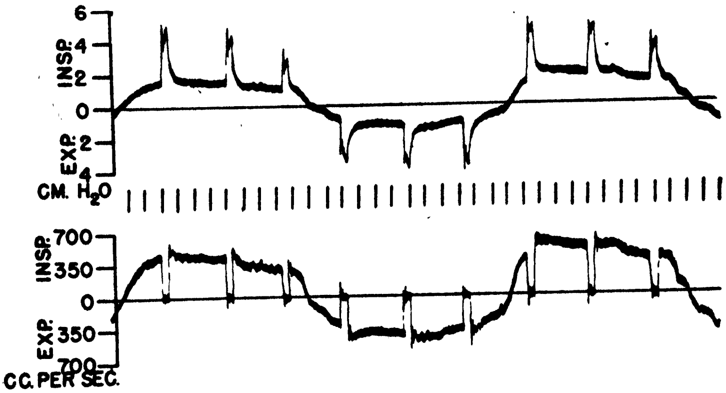
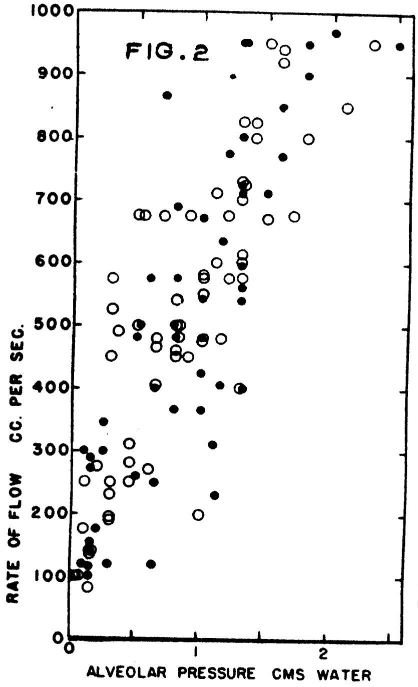


Fig. 1. Tracing of simultaneous records of pressure at the mouth (*upper curve*) and rate of flow (*lower curve*). Time intervals are 0.2 seconds. Records obtained by use of strain gage pressure transmitter.

None of the subjects reported any unpleasant sensations while interruptions were being performed. Comparison of records taken with and without interruption indicate no marked disturbance in the normal breathing pattern.

Measurements of the records yielded data relating alveolar-mouth pressure gradients to rate of flow. The relationship obtained for one subject is shown in figures 2 and 3. In all, 21 subjects were examined in this fashion and the data for each subject were similarly plotted. A curve was drawn freehand through each set of data, and in figure 4 the curves for all individuals are shown.

Although a definite relationship appears to exist between alveolar pressures measured by this method and the simultaneously measured rates of flow, one may ask whether this method really measures the alveolar pressure. With this question in mind a model experiment was performed. A balloon was placed in a Drinker respirator and connected in series with a resistance of gauze sponge to the apparatus through a pipe in the respirator wall. A small tube leading from the inside of the balloon was connected to an optical manometer. In this fashion the pressure within the balloon could be recorded directly. The respirator was sealed and the pump turned on so as to artificially ventilate the balloon. The 'alveolar' pressure of the balloon by the method of interruptions and by the direct method was recorded on the same film. Records of the sort shown in figure 5 indicate good agreement between the two methods. Admittedly the model is not a perfect imitation of the human lung, but it seems sufficiently similar in principle to give one confidence in the values obtained by the method of interruptions. Vuilleumier (2) also describes model experiments which indicate the validity of the method of interruptions.



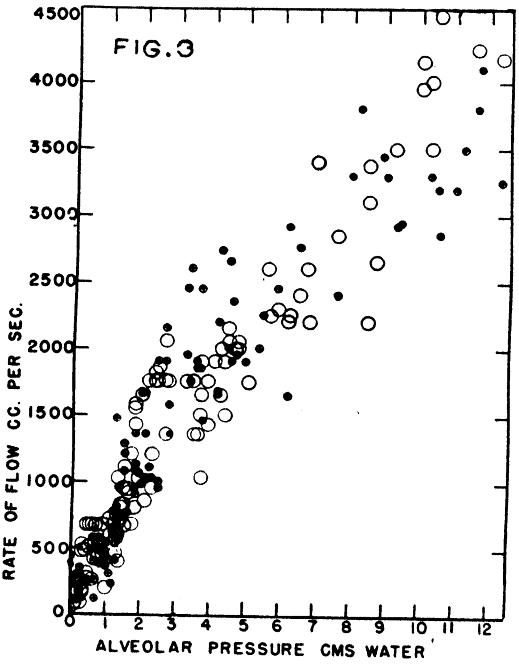


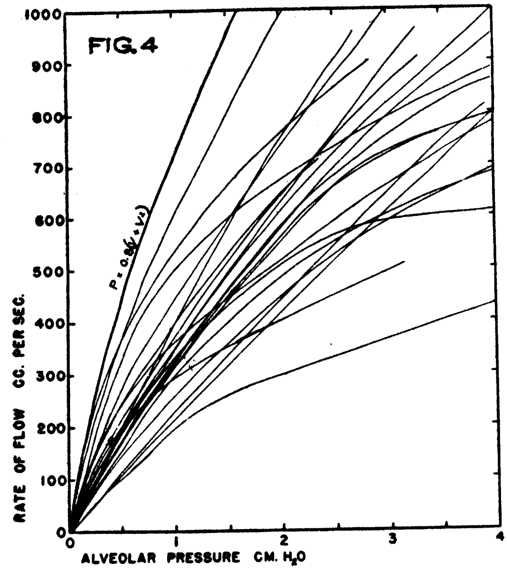
FIG. 2. RELATIONSHIP BETWEEN RATE OF FLOW AND ALVEOLI-MOUTH PRESSURE GRADIENT obtained for one subject. *Open circles* represent values obtained during inspiration, *solid circles*, during expiration.

FIG. 3. RELATIONSHIP BETWEEN RATE OF FLOW AND ALVEOLI-MOUTH PRESSURE GRADIENT for one subject. This figure includes the data shown in figure 2 but is extended to show higher rates of flow.

DISCUSSION

The data have been plotted with rate of flow as a function of alveolar pressure. The resistance of the respiratory tract to air flow is represented by the reciprocal slope of the curve fitting these points. According to the theoretical reasoning of Rohrer (1), the pressure drop along the respiratory tract is due to:

1. Viscous resistance with the pressure drop varying linearly with rate of flow.
2. Resistance due to changes in direction of flow (as in branching) and changes in x-sectional area of the airway with the pressure drop proportional to the



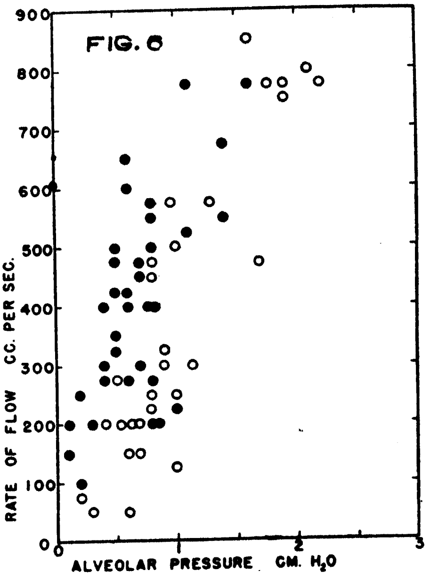


FIG. 5

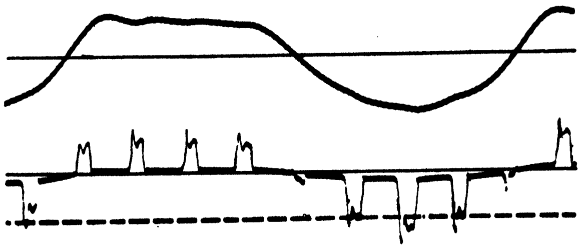


FIG. 4. RELATIONSHIP BETWEEN RATE OF FLOW AND ALVEOLI-MOUTH PRESSURE GRADIENT for 21 subjects. Rohrer's theoretical curve is shown for comparison. Since most of the subjects did not exceed a velocity of 1000 cc. per second, no higher values are shown.

FIG. 5. SIMULTANEOUS RECORDS OF PRESSURE in a balloon as measured directly (*upper curve*) and by the method of interruptions (*lower curve*). Time intervals shown at bottom are 0.2 seconds.

FIG. 6. RELATION OF RATE OF FLOW to alveolar pressure at two different lung volumes. *Solid circles* represent subject breathing with lungs as empty as possible; *open circles*, subject breathing with lungs as full as possible.

square of the rate of flow. This type of resistance is almost entirely confined to the upper airways (larynx and distal structures).

3. Resistance due to turbulence with the pressure drop varying with approximately the square of the rate of flow.

From measurements on cadavers Rohrer concluded that the critical velocity for transformation from streamline to turbulent flow would not be attained until the flow exceeded five liters per second, a rate that is seldom reached except in coughing. For any ordinary breathing, therefore, only the first two of the above factors would contribute to the pressure drop, and the equation relating pressure to rate of flow should have the form $P = K_1 V + K_2 V^2$.

The value of each of these constants, as determined by Rohrer from anatomical measurements, is about 0.8, so that the equation becomes $P = 0.8 (V + V^2)$, when P is expressed in centimeters of water and V in liters per second.

The curve for this equation has been plotted in figure 4 for comparison. All individuals show a higher resistance than the equation predicts, but it appears that an equation of this form would fit most of the data reasonably well, although the constants would have to be adjusted for each individual as would be expected.

The mean pressure for a flow of 250 cc/sec. is 0.7 cm.H_2O (range of 0.3 to 1.4 cm.H_2O), and for a flow of 500 cc/sec. is 1.8 cm. H_2O (range of 0.8 to 5.0 cm.H_2O). These figures agree well with values measured from the sample curves published by Vuilleumier. Values obtained by Neergaard and Wirz, using this method on three normal individuals, are about twice as great, but, judging from the form of the recordings shown by these authors, their method of interrupting was not sufficiently abrupt.

The scatter of the data for each individual (typically shown in figures 2 and 3) is probably due not only to experimental error in the measurement of the rates of flow and pressures but also to real physiological variations, especially at the high rates of flow. Slight changes in the configuration of the glottis and in the position of the tongue and other structures of the throat could appreciably alter the alveolar pressure required for a given rate of flow.

The results do not indicate any consistent difference between the resistance during inspiration and that during expiration. This is contrary to the observations of Neergaard and Wirz (3), who found that on three subjects expiratory resistance was greater than inspiratory. However, they were using the method of intrapleural pressure measurement and their values include the viscous resistance of the lung tissue. Moreover, their subjects were not normal, two of them having bronchial asthma and one tuberculosis.

Certain sources of error in the method must be mentioned in appraising the results of the present study. As stated above, the justification for equating the equalized pressure following interruption to the alveolar pressure is that the dead space volume is negligible as compared to the alveolar volume. Actually our apparatus has a dead space of about 650 cc. This would make the measured alveolar pressures lower than the actual by about 15 to 20 per cent, depending on the alveolar volume which, of course, varies from one individual to another.

The effect of voluntarily varying the alveolar volume has been tested on one individual by having him breathe with his lungs as full of air as possible and then with his lungs as empty as possible. The results are shown in figure 6 and indicate that the highest apparent alveolar pressure for a given rate of flow occurs when the lungs are most inflated, the lowest when the lung volume is low. Presumably these apparent differences in alveolar pressure are due in part at least to the difference in the size of the alveolar volume in relation to the dead space in the two conditions. This effect could be minimized by use of an apparatus with a small dead space.

Another factor, however, would tend to make the measured values somewhat too high and would compensate for the error just discussed. After the moment of complete occlusion a finite time (estimated by Vuilleumier to be in the order of 1/200 sec.) is required for the pressure in the system to become equalized. During this time the chest continues to move, thus raising the pressure above that which existed at the moment of interruption. The magnitude of this error will depend largely on the rate at which the chest is moving at the moment of interruption.

In spite of the sources of error mentioned above, this method for the estimation of alveolar pressures should, because of its relative simplicity and painlessness, prove useful in the investigation of both clinical and physiological problems in the mechanics of respiration.

SUMMARY

1. Measurements of alveolar pressure and simultaneous rate of flow of respired air of 21 human subjects are presented.

2. The data for each subject can be described by an equation of the form $P = K_1V + K_2 V^2$, where P is the alveolar pressure and V is the volume rate of flow. The constants K_1 and K_2 differ for different individuals.

3. In the average subject an alveolar pressure of 1.8 cm.H_2O is required to produce a flow of 500 cc. per second.

REFERENCES

1. Rohrer, F. Pflügers Arch. ges. Physiol. **162:** 225, 1915.
2. Vuilleumier, P. Z. klin. Med. **143:** 698, 1944.
3. V. Neergaard, K. and K. Wirz. Z. klin. Med. **105:** 52, 1927.
4. Bayliss, L. E. and G. W. Robertson. Quart. Jour. Exp. Physiol. **29:** 27, 1939.

REVERSIBLE CELLULAR PERMEABILITY ALTERATIONS IN DISEASE. IN VIVO STUDIES ON SODIUM, POTASSIUM AND CHLORIDE CONCENTRATIONS IN ERYTHROCYTES OF THE MALARIOUS MONKEY[1]

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Investigations recently reported from this laboratory have furnished strong inferential evidence of altered permeability of tissue cells in simian malaria (1–4). It has been shown *a*) that the volume of fluid available for the dilution of the foreign ion SCN progressively increases in monkeys fatally infected with *P. knowlesi*, *b*) that SCN volumes increase until they approach or become equal to the estimated total body water (64–65 per cent of the body weight) in terminal stages of the disease and *c*) that the SCN content of striated muscle, cardiac muscle, liver and spleen increases as the disease progresses.

Since these changes are associated with conditions in which there is no evidence of accelerated loss of SCN from the body or increased destruction or conversion of SCN, the inferences are strong that in simian malaria the foreign ion SCN ceases to be primarily an extracellular anion; that it progressively tends to enter cells, and that a determination of its dilution eventually is a measure of the total body water. It has likewise been shown that such altered cell membrane permeability is reversible following chemotherapeutic intervention although the return to normal is a slow process requiring several months (1).

In the normal animal or man the foreign ion SCN is distributed in the fluids approximately as is sodium and chloride and its dilution, therefore, under such conditions is an approximation of the 'extracellular' fluid volume (5). If, in malaria or other disease, SCN ceases to be an extracellular anion, then it might be expected that alterations in the distribution of the native ions, Na, K and Cl, would likewise occur. The work to be described elucidates in part these native ionic shifts which accompany fatal simian malaria.

METHODS

Control determinations of Na, K and Cl concentrations of the whole blood, plasma, erythrocytes and the hematocrit values of 15 normal *Macacus mulata* monkeys were made after which each animal was infected with *P. knowlesi* malaria by blood inoculation from an animal bearing a chronic infection. A total of 70 serial determinations of these ionic levels were subsequently made at approximately 24-hour intervals until death from malaria occurred.

Sodium and potassium concentrations in blood, plasma, red cells and, in some instances, urine were measured by the flame photometric method of Overman and Davis (6). Chlorides were determined by a modification of the method of Volhard (7). The cell ionic levels were determined on non-parasitized erythrocytes obtained by centrifugation for one hour at 2300 G. Hematocrits were measured by the Wintrobe tube method.

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[1] The research reported in this paper has been supported in part by a Research Grant-in-Aid from the U. S. Public Health Service.

Blood samples for analysis were withdrawn by needle puncture from the femoral artery into an oiled heparinized[2] syringe and were delivered under oil into the collection tube to prevent the 'chloride shift'. The femoral artery was not exposed for sampling and hematomata were prevented by finger stasis and massage.

RESULTS

Determinations of the ionic levels were made at parasitemias ranging from zero to 123 per cent (i.e., some of the cells containing more than one parasite) and at hematocrit levels ranging from 50 to 7.0 per cent. Multiple determinations at each parasitemic and/or hematocrit level were desirable but not always feasible. Consequently, the results have been analyzed by grouping the data in a logical but arbitrary manner. It was found that while the ionic alterations which occurred were but poorly correlated (correlation coefficient = 0.35) with hematocrit changes, that there was a highly significant correlation (coefficient = 0.89) between the observed cellular ionic changes and parasitemia. Therefore, we have divided the determinations made in this series into 5 groups: (I) normal (control) values, (II) values obtained on animals which showed a positive blood smear for *P. knowlesi*, but had too few parasites to establish a definite count, (III) values obtained on animals which had definite parasite counts up to 5 per cent, (IV) values on animals with from 5 to 10 per cent parasitemia, and (V) values obtained on animals in which more than 10 per cent of the erythrocytes were parasitized.

Whole blood. Referring to table 1 and figure 1 it can be seen as was postulated that the levels of Na, K, and Cl in blood reflect the progressive anemia. Thus, as the hematocrit value declines, the Na level of whole blood rises and the K concentration decreases. While the average hematocrit fell from 42.2 to 21.4 per cent, average whole blood Na increased 27.7 mEq/l., the average Cl concentration increased but 4.2 mEq/l., and blood K was reduced 22.6 mEq/l. on the average.

Plasma. Although the alterations in plasma Na, K, and Cl concentrations generally fail of statistical significance (table 2), reference to figure 2 will illustrate the trends. In general, the tendency is for plasma Na and Cl to be reduced and for plasma K to rise. The failure of these values to reach statistical significance lies, we believe, in the fact that the kidney remains highly capable of regulating the plasma ionic levels until the terminal stages of the disease.

Erythrocytes. Although strong inferential evidence that the cells of the malarious monkey become increasingly permeable to the foreign ion SCN has been previously presented (1, 4), we felt obliged *a*) to seek evidence of *native* ionic

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[2] Heparin used in this study was kindly supplied by Roche-Organon, Inc., Nutley, New Jersey.

TABLE 1. Comparison of Na, K and Cl levels in whole blood, plasma and erythrocytes and hematocrit values in groups I, II, III, IV and V[1]

GROUPS	WHOLE BLOOD			PLASMA			ERYTHROCYTES			HEMATOCRIT
	Na	K	Cl	Na	K	Cl	Na	K	Cl	
I (n = 13)										
Range	86.1–109.1	42.3–61.5	81.7–108.0	136.1–176.9	3.3–5.1	103.4–116.3	12.6–27.4	98.7–131.3	54.9–69.2	37.1–49.7
Average	99.1	52.9	91.6	156.6	4.2	110.2	18.9	116.6	64.5	42.2
S.D.	6.5	4.8	8.0	12.5	0.7	4.6	4.7	9.7	7.5	3.1
S.E.	1.97	1.45	2.67	3.61	0.19	1.51	1.33	2.80	2.83	0.89
II (n = 5)										
Range	105.7–112.2	37.7–51.5	86.8–102.0	153.0–173.9	3.2–4.8	104.2–116.3	13.7–23.1	109.0–120.0	51.3–72.9	34.3–40.1
Average	107.9	45.1	96.0	159.1	4.0	110.6	18.3	115.5	66.3	37.4
S.D.	2.3	5.1	5.6	7.6	0.6	4.5	3.2	6.3	7.8	2.2
S.E.	1.13	2.55	2.79	3.79	0.94	2.26	1.59	3.64	3.88	1.10
III (n = 7)										
Range	96.5–118.6	36.9–47.7	82.6–98.9	147.0–169.6	3.1–4.8	95.7–110.9	12.6–23.1	111.3–116.6	51.0–69.4	31.0–36.6
Average	107.2	42.3	91.9	158.4	3.8	104.9	17.1	114.1	61.5	34.3
S.D.	6.9	3.2	5.4	6.9	0.53	5.9	3.4	1.9	7.0	1.7
S.E.	3.08	1.44	2.39	2.80	0.21	2.63	1.53	0.85	3.52	0.69
IV (n = 6)										
Range	117.8–140.9	12.8–40.5	90.3–114.0	149.0–173.9	4.0–5.2	103.1–110.8	14.6–30.4	96.7–121.5	62.0–90.6	11.2–34.5
Average	127.9	26.4	101.8	156.4	4.7	108.1	23.6	110.3	79.7	23.2
S.D.	9.5	10.1	8.4	8.7	0.44	3.2	5.2	10.6	12.6	8.8
S.E.	5.46	5.83	4.87	4.37	0.22	1.84	2.30	5.27	8.93	4.38
V (n = 13)										
Range	95.2–167.3	12.5–44.1	85.1–101.7	121.7–176.1	3.6–5.8	84.8–111.8	24.3–66.5	86.7–116.7	51.3–80.0	6.9–36.5
Average	126.8	30.3	95.8	150.5	5.0	100.4	39.3	102.8	70.9	21.4
S.D.	17.8	9.4	5.6	13.9	1.1	6.8	15.3	11.9	10.6	9.6
S.E.	5.62	3.13	2.12	4.03	0.36	2.04	4.83	4.51	5.31	2.88

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[1] See footnote to table 2; see also text.

shifts and *b*) to search for such changes in a position which is physiologically protected from direct renal regulation. The red blood cell was chosen for analysis because it can be secured practically free of 'extra-cellular' fluid, consequently obviating the difficulty of interpreting whether the ions measured reside inside or outside the cells.

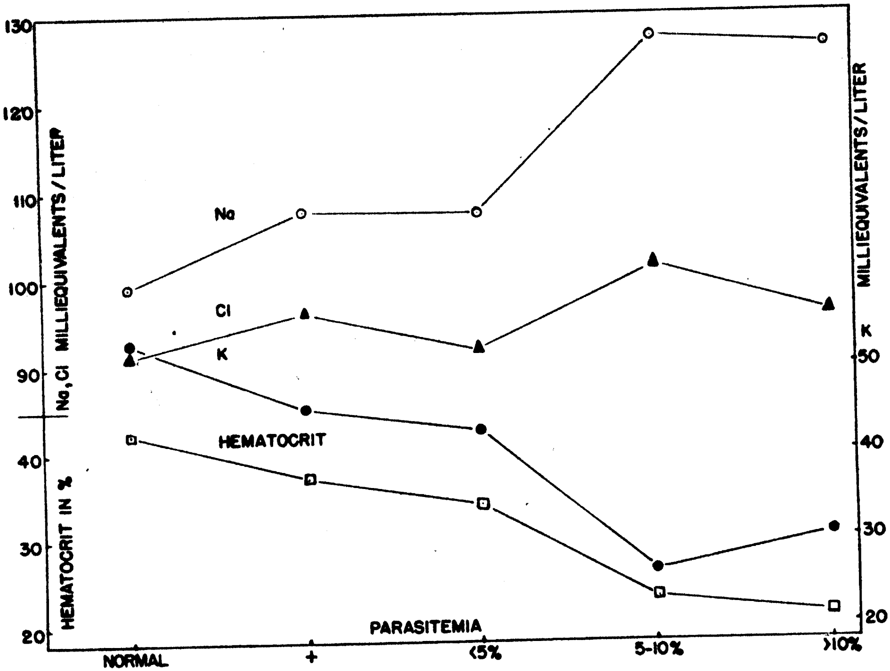

FIG. 1. RELATION BETWEEN WHOLE BLOOD NA, K AND CL CONCENTRATION, hematocrit values and parasitemia.

TABLE 2. STATISTICAL COMPARISON (*t* VALUES) OF IONIC LEVELS AND HEMATOCRIT IN GROUPS I, II, III, IV AND V

BETWEEN GROUPS	WHOLE BLOOD			PLASMA			ERYTHROCYTES			HCT
	Na	K	Cl	Na	K	Cl	Na	K	Cl	
I and II.......	3.88[1]	2.65[1]	1.14	0.48	0.57	0.15	0.29	0.20	0.39	3.38[1]
I and III......	2.21[1]	5.17[1]	0.08	0.39	1.37	1.74	0.89	0.85	0.68	6.99[1]
I and IV.......	5.04[1]	4.41[1]	1.83	0.04	1.72	0.88	1.77	1.06	1.62	4.25[1]
I and V.......	4.66[1]	6.75[1]	1.27	1.15	2.00	3.45[1]	4.07[1]	2.65[1]	1.06	6.91[1]

[1] These values are significant at the 1 per cent level.

Group I = normals; *Group II* = animals with positive blood smear (no count); *Group III* = animals with definite count, but less than 5 per cent of cells parasitized; *Group IV* = animals with parasite counts between 5 and 10 per cent; *Group V* = animals with greater than 10 per cent parasitemia.

Since the density of the parasitized erythrocyte is considerably lower than that of the non-parasitized cell, separation of invaded cells can be obtained by centrifugation. The erythrocytic samples for ionic samples were drawn from the bottom of the centrifuged sample and hence are, in general, non-parasitized cells.

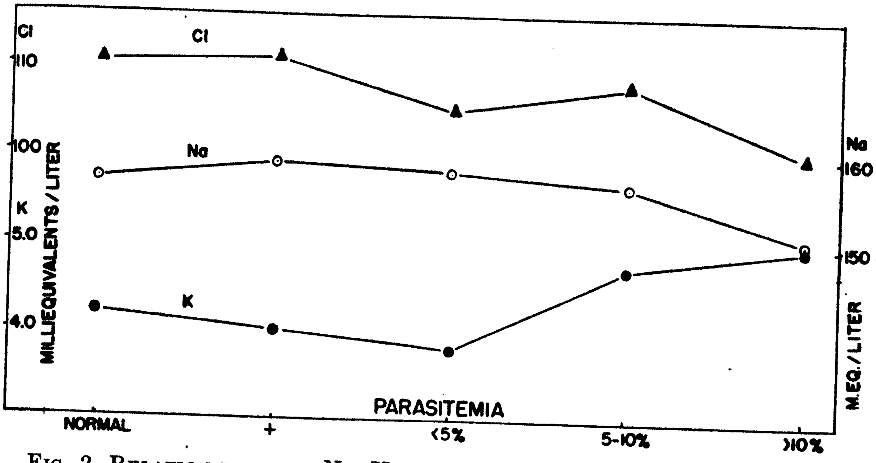

FIG. 2. RELATION BETWEEN NA, K AND CL LEVELS of plasma and parasitemia

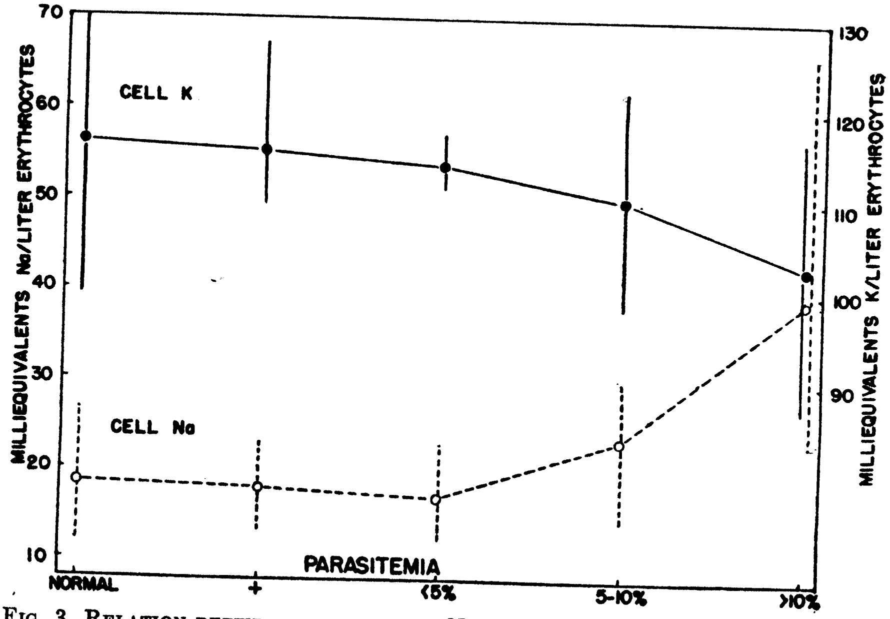

FIG. 3. RELATION BETWEEN ERYTHROCYTE NA CONCENTRATION and erythrocyte K concentration and parasitemia. Vertical lines indicate ranges.

It can be seen (fig. 3) that the average Na content of non-parasitized red blood cells increases from an average normal value of 18.9 to 39.3 mEq/l. before death.

And although this reported average increase is greater than 100 per cent, several cases were encountered in which the increase was even larger.

Concomitantly there is a reduction in the concentration of erythrocyte K concentration (fig. 3). The average reduction was 13.8 mEq/l. as compared to a Na increase of 10.4 mEq/l.

We have previously shown that altered cellular permeability in the living malarious animal as revealed by alterations in the distribution of SCN were reversible following chemotherapeutic intervention (1). Consequently, attempts were made to demonstrate whether the *native* ionic shifts reported here were reversible. Figure 4 illustrates one such case. Control determinations on this animal showed *a*) a cell Na concentration of 13.7 mEq/l., *b*) cell K concentration of 131.3 mEq/l., and *c*) cell Cl level of 64.8 mEq/l. Following the induction of *P. knowlesi* infection, cell Na and Cl values increased 16.4 and 31.9 mEq/l. respectively, and cell K fell 34.8 mEq/l. by the time a 7.7 per cent parasitemia developed. At this time sulfadiazine was administered (five doses, 3 ml. of 0.5 per cent solution of the Na salt over a three-day period). The parasitemia gradually decreased until the animal showed only a positive thick blood film. At this time (five days after beginning chemotherapy) the cell Na was reduced again by 14.8 mEq/l., cell Cl fell 28.1 mEq/l. and cell K rose 16.8 mEq/l. Although none of the cell values obtained at this time are strictly at control levels, it is apparent that reversal of each of the native ionic alterations is accomplished following chemotherapy.

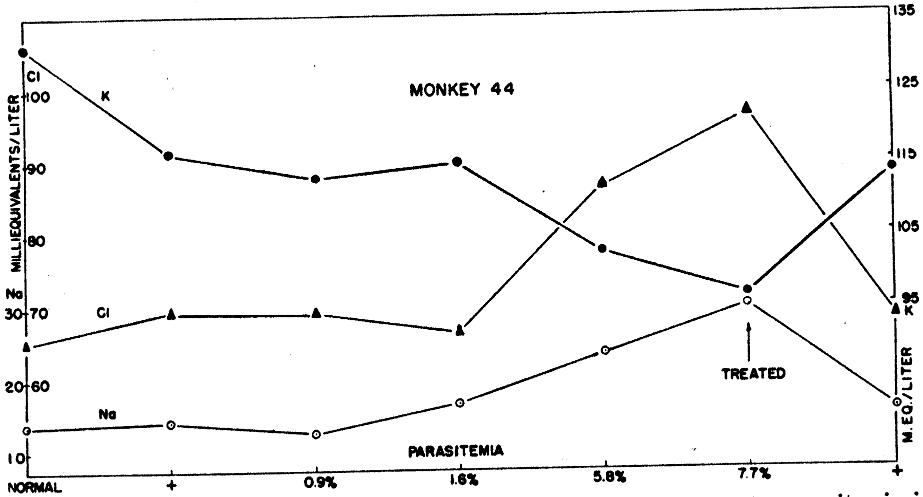

FIG. 4. RELATION OF ERYTHROCYTE NA, K AND CL CONCENTRATION to parasitemia in *monkey 44*. (*Note:* This animal was treated with sulfadiazine when the parasitemia reached 7.7 per cent.)

Urinary excretion of Na, K and Cl has been studied serially throughout the malarious course in four animals. Variation from animal to animal and the small number studied precludes statistical presentation of this data. However, data illustrating the alterations in urinary excretion of these ions in *Monkey 22* are qualitatively and quantitatively typical of the group. In this animal the total urinary Na and Cl output fell from normal values of 480 and 640 mEq., respectively, to 10 and 40 mEq. by the time the animal had an 84 per cent parasitemia. Concomitantly, urinary K rose from 420 to 740 mEq. and only fell again as the animal became oliguric. Thus, the postulate that the reason for failure to find highly significant alterations in plasma levels of Na, K, and Cl is the fact that the kidney continues to regulate these levels seems to be borne out. As cellular permeability is altered by the disease and Na and Cl tend to enter cells, the resulting tendency for the plasma levels to be reduced is compensated for by increased renal tubular reabsorption of these ions. Hence the striking reduction in urinary output of Na and Cl. And, as K comes out of cells, the tendency for plasma K to rise is likewise renally compensated for by an increased excretion of K. When serious oliguria supervenes and the excretion of K consequently is reduced, *the K level of the plasma is increased* (fig. 2).

The total collected fluid from the metabolism cages on the succeeding five days reported was 90 cc., 115 cc., 188 cc., 66 cc., and 36 cc., respectively. Urinary volumes have not been graphed, however, since the animals may spill drinking water in the cage which would make the volumes erroneous. We have, therefore, measured Na, K and Cl concentration of the total collected fluid and expressed the results as total rather than as concentration.

DISCUSSION

Attempts to show alterations in the ionic levels of body fluids in malaria have been made previously (8–13). In general the failure to show striking changes may have been due to two factors: *a*) the study of *plasma* levels of ions only in a condition in which the kidney is not seriously functionally impaired (until terminal stages) will, of course, fail to reveal large alterations; and *b*) the almost exclusive use in the past of the human malarial host, who may lose large amounts of ions and water in sweat, has complicated the interpretation of results on both plasma levels and urinary excretion of ions. To our knowledge, cellular ionic levels (Na and K) have never been studied previously in malaria.

Although the present study deals only with erythrocytes, the normal permeability of which is undoubtedly different from that of muscle and other tissue cells, the inferential evidence is strong that considerable alteration in tissue cellular milieu likewise occurs.

During the course of fatal simian malaria the foreign ion SCN comes to be distributed in the entire water mass of the organism (1). Since Na and SCN are normally largely confined to the extracellular space, one might expect a similar derangement of Na distribution in malaria. The experiments cited show this to be true of at least the erythrocyte.

The alterations reported here in intracellular ionic levels could not be explained on the basis of water shifts into or out of the red blood cell. Water shifts may well occur and if so, depending upon their direction, one or the other of the illustrated ionic changes would be emphasized. Since in early simian malaria the Na and K alterations in cells are unidirectional (fig. 3), it might be postulated that at this time the cells take up water. Later, however, when the Na and K concentration changes in cells are in opposite directions, water shifts cannot account for the observed phenomena.

Lack of significant correlation between these intracellular ionic shifts and hematocrit values suggests that they are not associated with the increasing anemic anoxic state. Further, the high correlation (coefficient = 0.89) of ionic alteration with parasitemia argues that such changes may be the result of *a*) toxic substances formed as the result of parasite metabolism or the influence of parasite on host metabolism or *b*) influences of the paroxysmic or febrile course upon the vital function of cellular membranes. Recent experiments in human therapeutic malaria, where the paroxysmic course can be accurately followed, indicate that cellular ionic shifts are best correlated with the *number of paroxysms* and not with parasitemia, height of fever, number of hours of fever or days of disease. Unfortunately it has not been possible to follow the febrile course in malarious monkeys.

In general, at high parasitemic levels (40 per cent or more of the red cells invaded) the monkey is not readily saved by chemotherapeutic intervention even though such intervention invariably reduces the parasitemia to levels which should result in amelioration of clinical symptoms. It would seem, then, that late in the disease some factor or factors other than parasitemia must be responsible for the continued downward trend. We believe an important one of these factors is an altered cellular permeability which leads to a drastically altered intracellular ionic anatomy. Whether physiological intervention based upon attempts to return the ionic distribution to normal at this time would result in improvement is, as yet, unknown.

SUMMARY

Although the plasma levels of Na, K and Cl are but little altered during the course of fatal *P. knowlesi* infections in monkeys, relatively enormous changes are found in non-parasitized erythrocytes.

Red blood cell Na is increased on the average of 100 per cent before death; erythrocyte K levels concomitantly fall and Cl levels rise.

Urinary excretion of Na and Cl decrease while that of K rises until late in the malarious course when the animals become oliguric.

Reversal of all the native ionic shifts is effected following chemotherapeutic intervention early in the malarious course.

A discussion of the etiology of these changes and their possible importance is presented.

The author is indebted to Mrs. Virginia Fogg, Department of Health and Safety, Tennessee Valley Authority, for making daily parasite counts on the animals used in this study and to Dr. J. P. Quigley for advice and criticism of the manuscript.

REFERENCES

1. **Overman**, R. R. J. Lab. Clin. Med. **31**: 1170, 1946.
2. **Overman**, R. R. Federations Proc. **6**: 174, 1947.

3. Overman, R. R. Communications, 17th Int. Physiol. Cong., Oxford, 1947.
4. Overman, R. R., and H. A. Feldman. J. Clin. Invest. **26**: 1049, 1947.
5. Crandall, L. A. Jr., and M. X. Anderson. Am. J. Digest. Dis. and Nutrition **1**: 126, 1934.
6. Overman, R. R., and A. K. Davis. J. Biol. Chem. **168**: 641, 1947.
7. Peters, J. P., and D D. Van Slyke. Baltimore, Quantitative Clinical Chemistry: Methods. 1932. 829 ff.
8. Pinelli, L. Riv. di malariol. **3**: 310, 1929.
9. Velick, S. F., and J. Scudder. Am. J. Hyg. **31**: 92, 1940.
10. Zwemer, R. L., E. A. H. Sims, and L. T. Coggeshall. Am. J. Trop. Med. **20**: 687, 1940.
11. Flosi, A. Z. Rev. Clin. de Sao Paulo **16**: 1, 1944.
12. Morin, M., H. Bader, E. Monnier, and P. Moreau. Bull. Soc. Path. Exot. **27**: 136, 1934.
13. Emerson, K., Jr., and V. P. Dole. Bumed News Letter **4**: 5, 1944.

CAUSES OF SKIN COOLING IN PRESSURE BREATHING, DEEP INSPIRATION AND DEEP EXPIRATION[1]

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Transient peripheral vasoconstriction has been observed incident to positive pressure breathing (P.P.B.) (1, 2). It was considered possible that this vasoconstriction originated from stretch receptors located in the lungs or chest wall. Therefore it seemed of interest to discover what effect a pneumatic vest would have during P.P.B. since the vest largely prevented abnormal expansion of the chest. The results are also of interest from the more general point of view of the vasoconstrictor reflexes which originate from negative pressure breathing, deep inspiration and deep expiration.

METHODS

Skin temperature was measured with very light copper-constantan junctions which were attached to the skin with Scotch tape or collodion. In some experiments a separate thermocouple was attached to each finger and these were read in rotation each in series with its own cold junction in ice water. For the recording experiments several such pairs of junctions were used in series and the potential of the whole series was recorded on an optical kymograph with an HS Leeds Northrup galvanometer. This arrangement gave 4 to 5 cm. deflection for one degree Centigrade. Efforts were made to measure skin temperature of the toes simultaneously, but it was found that the temperature of the fingers was far more sensitive to vasomotor influences of this type. Various types of applicators for holding a series of thermojunctions against the skin were also tried but they proved unsatisfactory either because of their large heat capacity or because the pressure which they exerted occluded the vessels which were under observation. The method which we adopted gave no pressure against the skin, minimal heat capacity and a minimal protection against the temperature changes in the environment.

In addition to the skin temperature we used a finger plethysmograph similar to that described by Fenn and Chadwick (1). This included a pneumatic cuff around the base of the finger so that rates of blood flow could also be recorded optically. A boot plethysmograph was also used for some purposes similar to that described by Fenn, Otis, Rahn and Chadwick (2).

Negative pulmonary pressures down to -20 cm. of water were obtained by connecting a suction pump to a large tank and allowing the subject to inspire through a pressure face mask (A-13 U. S. Army Air Force) connected to the tank.

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[1] This work was done under contract between the University of Rochester and the Air Materiel Command at Wright Field.

Expired air was eliminated through a Linde air-loaded valve in the mask. Negative pressure-breathing was administered continuously for approximately 10 minutes until a stable skin-temperature was reached.

Positive pressures of 20 cm. of H_2O were obtained by use of a Pioneer pressure demand valve on a high pressure (40 p.s.i.) air line, connected to an A-13 face mask worn by the subject, the expiratory port being in the mask as described above.

P.P.B. was administered continuously during two periods of approximately 10 minutes each (until a constant skin temperature was established) with an intervening rest period of 10 minutes. An inflated pneumatic vest was worn during one of the two periods of P.P.B.

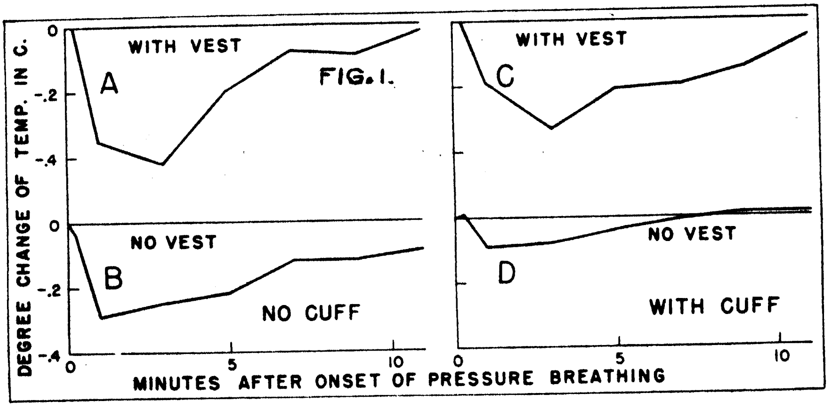
The pneumatic vest used for these experiments was supplied by the U. S. Navy and was connected into the inspired air line in parallel with the subject so that the vest was always inflated to the same pressure as the lungs. It served, therefore, to prevent any abnormal expansion of the chest during P.P.B. but did not interfere with normal breathing. On some of these same subjects an arm cuff was applied around the middle third of the upper arm on the same side as the thermocouples. This was inflated to 70 mm. Hg 15 minutes prior to onset of P.P.B. and kept inflated at that level for the remainder of the run. At this pressure the cuff prevents venous pressure changes which originate in the thorax from being transmitted back through venous channels to the hand, and presumably maintains the distal venous pressure at a constant level. In five subjects the vest was kept inflated to the same pressure that obtained in the mask during the first period of pressure breathing, and in six subjects it was inflated during the second period of pressure breathing. This compensated for any errors introduced by fatigue when comparing P.P.B. effects without vest to P.P.B. effects with vest.

Young healthy males were used in all experiments. In all cases the subjects were allowed to rest for at least 20 minutes prior to the experiments so that skin temperature fluctuations could approach an equilibrium value. It is worth noting that in the case of deep expiration the subjects were instructed to prevent a deep inspiration from following their forced expiration, and the reverse with deep inspirations.

RESULTS

Positive pressure breathing. A summary of the effects of P.P.B. on skin temperature is shown in figure 1. In all of 11 subjects tested P.P.B. produced rapid cooling of the fingers, reaching an average maximum intensity of −0.3°C. below normal in an average time of one minute after onset of P.P.B. Thereafter the skin temperature gradually returned toward normal until by the seventh minute (average) a new base line slightly cooler than normal was established (fig. 1B). This confirms the report of Bateman and Sheard (3). The range of individual responses can be determined from figures 2A and 2C.

In general the use of an inflated vest increased the magnitude of skin cooling which resulted (compare figs. 1A and 1B). This was true in all five subjects



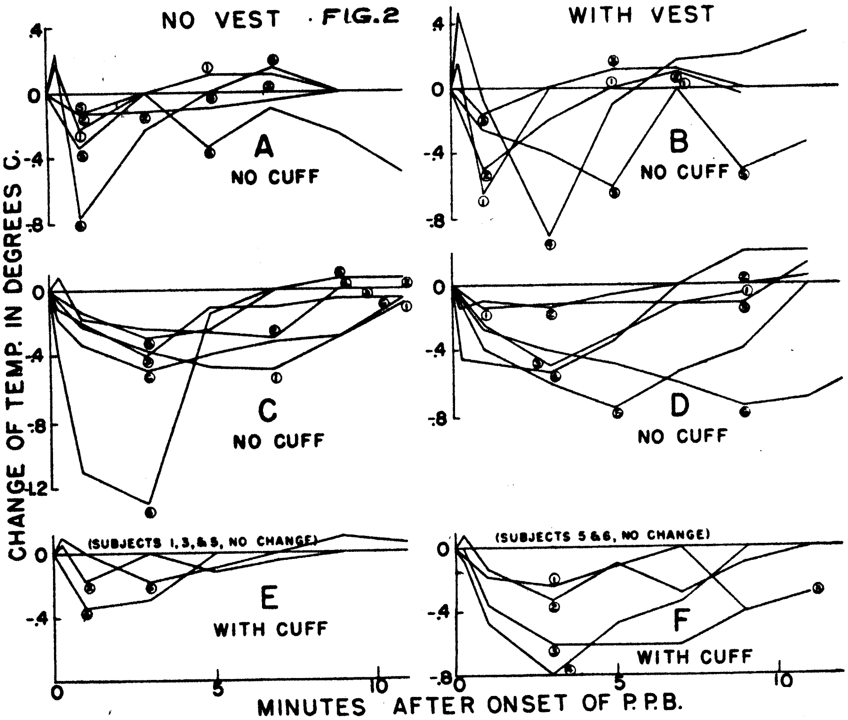


FIG. 1. EFFECT OF P.P.B. ON SKIN TEMPERATURE, with and without a vest; *C* and *D* with and *A* and *B* without a cuff. The graphs represent the average of all the results in each category. P.P.B. started at zero minutes and continued throughout in each case. The temperature scale represents deviation from the base line.

FIG. 2. EFFECT OF CONTINUOUS P.P.B. ON SKIN TEMPERATURE. Three series of experiments, each on five or six subjects, are represented by *a*) groups *A* and *B*, *b*) groups *C* and *D*, *c*) groups *E* and *F*. Groups *B*, *D* and *F* wore an inflated vest; groups *A*, *C* and *E* did not wear a vest. Groups *B*, *C* and *F* preceded groups *A*, *D* and *E*, respectively, with a 10-minute intervening rest period. The numbers on the graphs refer to subjects. Subject No. 1 in *A* is the same as subject No. 1 in *B* but not necessarily the same as subject No. 1 in *C* or *E*. Figures *C* and *D* are similarly related, as are *E* and *F*. P.P.B. started in all cases at zero minutes.

in which the experiment was so run that the period with the vest was tried before the period without the vest (compare figs. 2A and 2B). When the order was reversed (i.e., the vest inflated during the second period of P.P.B.), the vest enhanced the skin cooling in three out of six subjects, indicating perhaps, a fatigue effect (compare figs. 2C and 2D).

A pneumatic cuff was applied to the upper arm above the thermocouples in six cases. In three out of these six cases, no change with P.P.B. was demonstrable; in the remaining three there was a slight cooling effect (fig. 2E). Figure 1D shows the average curve. In these same six subjects, when the vest was inflated together with the arm cuff, the skin cooling was magnified materially in four cases. Compare figures 1C with 1D and 2E with 2F. In eight of the 34 trials of P.P.B., an initial warming effect was noted during the first 15 seconds (see fig. 2, 3A and 3B).

Typical galvanometer records of skin cooling with pressure breathing are shown in figures 3A and 3B, with and without the vest, respectively. The existence of vasoconstriction in the finger was confirmed by records taken with the finger plethysmograph, examples of which are shown in figures 3D and 3E.

In the experiments from which the average curves of figure 1 were taken, there is probably not enough data to establish the relative positions of all the curves with certainty. Since, however, in all cases without a cuff there was cooling of the skin, the presence of a vasoconstriction seems established beyond any doubt. Furthermore, it seems certain that the addition of a pneumatic vest to keep the chest movements in the normal range does not eliminate but seems to enhance the magnitude of the reflex vasoconstriction. This seems to prove clearly that the reflex is not initiated by an expansion of the chest.

Our work with P.P.B. in four subjects confirmed the diminution of foot volume with P.P.B. (during partial occlusion by a leg cuff) reported by Fenn and coworkers (2); in four other subjects a diminution of finger volume was also demonstrated in all cases when a finger cuff had been previously inflated to 60 mm. Hg (see fig. 3D). One subject in each group wore an inflated vest without apparent effect upon the response (see fig. 3E).

Negative pressure breathing. In five subjects skin cooling varied from −0.1°C. to −0.6°C. below normal and lasted from one to four minutes. In two of these subjects finger volumes were recorded on the same film and a simultaneous diminution of finger volume was shown to occur. A record of such an experiment is shown in figure 3C. It is significant and perhaps surprising that the effect of negative pressure is the same rather than the opposite of the effect of positive pressure.

Deep inspiration. Early in these experiments the vasoconstrictor effect of a deep inspiration and later of a deep expiration was noticed. Repeated records were taken by the various methods described above in the hope of learning more about the nature of these respiratory vasomotor reflexes. The results of a deep inspiration may be summarized as follows.

In five subjects skin cooling varied from 0.15°C. to 0.57°C. below normal and lasted from 1.5 to 5 minutes. A typical record is shown in figure 4A. In six other subjects, finger volume diminished in all cases, the diminution lasting from 0.5 to 2.5 minutes. In four subjects, rates of finger blood flow (cc/sec.) were measured by the method of Fenn and Chadwick (1) and they diminished 30 to 90 per cent, returning to normal within 1.5 minutes. A record of such an

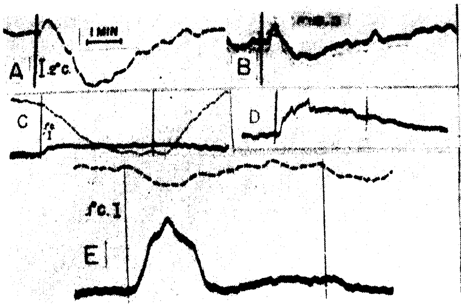


FIG. 3.

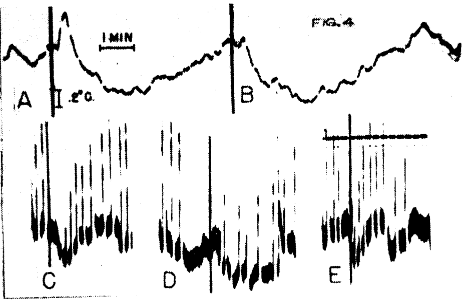


FIG. 4.

FIG. 3. *3A*. SKIN TEMPERATURE RECORD OF P.P.B. with vest inflated showing cooling (downward deflection). P.P.B. turned on at vertical line. *3B*. Skin temperature record of P.P.B. without vest. P.P.B. started at vertical line. Same subject as in 3A; same time and temperature scale as in 3A. *3C*. Simultaneous skin temperature (dotted line) and finger volume recorded during negative pressure breathing, started at first vertical line and stopped at second vertical line. Cooling is represented by the downward deflection of the dotted line, and diminished finger volume is represented by the upward deflection of the solid line. *3D*. Finger volume record during P.P.B. without vest and with inflated finger cuff, starting at first vertical line and stopping at second, showing diminution of finger volume (upward deflection). In 3C and 3D, time scale is one half that of 3A. *3E*. Simultaneous skin temperature (dotted line) and finger volume curves (different fingers of same hand) recorded on same subject as 3D during P.P.B. with vest inflated. Finger cuff inflated only on finger producing the lower record. Note simultaneous cooling of skin (downward deflection of top line) and diminution of finger volume (upward deflection of bottom). Time scale as in 3A. Interruptions in records in *A*, *C* and *E* at 13.5 sec.

FIG. 4. *4A*. SKIN TEMPERATURE RECORD SHOWING COOLING (downward deflection) following a single deep inspiration at solid vertical line. *4B*. Continuous with 4A, showing skin cooling following a single deep expiration at solid vertical line. *4C*. Diminished rates of arterial blood flow in the finger and diminished finger volume following a single deep inspiration at solid vertical line. Rates of flow indicated by height of spikes above base line. Note that first attempt to record rate of flow after the deep inspiration indicates zero flow. The wide base line indicates finger volume diminution (downward deflection) occurring simultáneously with the diminished rate of blood flow. 4D and 4E are records similar to 4C but for a single deep expiration taken at the solid vertical line in each case. Again note the absent or nearly absent spikes directly following deep expiration. The time scale is constant for all records as indicated in *A*. Time intervals of 13.5 sec. indicated in *E*.

experiment is shown in figure 4C. Each upstroke starts at the moment when a pneumatic cuff around the base of the finger is inflated and terminates after a given interval of time a few seconds later. The height of the upstroke is then a direct measure of the rate of flow. The black line extending both above and below the base line marks the time of a single rapid deep inspiration. Following this the first inflation of the cuff (just before the first gap in the record) gave no filling at all. The second inflation gave a very small flow and successive inflations at intervals of 10 to 20 seconds revealed blood flow gradually returning to normal. There is also a simultaneous drop in base line, representing a diminution of finger volume. This tracing is an excellent demonstration of the dramatic vasoconstrictive effects of a deep breath. The drop in blood flow to almost zero confirms the statement of Mulinos and Shulman (11) that blood flow in the finger nail capillaries stops immediately following a deep inspiration.

A cuff was inflated to 60 mm. Hg around the experimental arm prior to the deep inspiration and maintained during the run in four cases. Skin temperature measured in one of these revealed skin cooling of 0.5°C. after a deep inspiration with recovery in five minutes. In the other three subjects finger volumes diminished with recovery occurring in 0.5 to 1 minute.

Deep expiration. In five subjects skin cooling after a deep expiration varied from 0.11°C. to 0.6°C. below normal and lasted from one to three minutes (see fig. 4B). In six other subjects finger volume diminished in all cases, the diminution lasting from 0.5 to 2.5 minutes. Measurements of the rate of blood flow in three subjects showed a diminution of 80–90 per cent and a return to the normal value within 0.5 to 1.5 minutes.

In two subjects foot volumes were measured. In one case foot volume diminished with both a deep inspiration and a deep expiration. In the second subject records of finger and foot volumes, recorded photographically on the same film, indicated simultaneous diminution of finger and foot volume following both deep inspiration and expiration and simultaneous recovery within 60 seconds.

It is interesting that the effects of deep inspiration and deep expiration are so similar in their reflex vasomotor effects. This finding does not confirm the report of Bolton, Carmichael and Stürup (10), who found that expiration had no effect on the finger volume. This discrepancy may possibly be due to differences between subjects or to different techniques of carrying out the deep expiration. Our studies have not been sufficiently exhaustive to settle this matter. It might be mentioned, however, that the case is somewhat parallel to positive and negative pressure breathing, both of which had the same reflex effect on the blood vessels. The similarity in the effects of expiration and inspiration is another indication that the size of the chest per se does not determine the nature of the reflex effect which is set up.

DISCUSSION

Perhaps the most surprising aspect of the results obtained is the fact that reflex peripheral vasoconstriction occurred after both positive and negative pressure breathing, and after both deep rapid inspirations and deep rapid expirations.

Transient skin cooling incident to P.P.B. was first noted by Bateman and Sheard (3). That this skin cooling is due to a reflex peripheral vasoconstriction seems reasonably well established by the demonstration of coincident transient diminution of pulse volume and of rate of peripheral blood flow (1, 2). The cause for the initial skin-warming effect is obscure, but it seems possible that it represents an initial rise in peripheral blood flow caused by the increased left heart output which occurs for a few seconds after the onset of P.P.B. As P.P.B. is applied the pulmonary blood pool is reduced because the increased intrapulmonary pressure not only impedes the systemic venous return but also forces blood out of the lungs. Thus the initial effect is to increase the left heart output at the same time that the right heart output is decreased. Some seconds later, however, balance is restored and the left heart output is diminished.

It might be argued that the diminished venous pressure gradient from periphery to heart which occurs with P.P.B. (4) is responsible for the skin cooling. In all probability it accounts for a portion of it, since we have demonstrated that the cooling effect of P.P.B. in the fingers is lessened by an inflated arm cuff (see fig. 1). But this cuff largely prevents transmission of pressure changes from thorax to finger, so it is readily seen that there is at least one other factor.

Cardiac output studies (6, 7) have revealed a slight decrease in both stroke and minute volumes in humans during P.P.B. Here again, it might be said that the reduced peripheral blood flow secondary to diminished cardiac output could explain the observed skin-cooling effect. However, arterial blood pressure in the arm (relative to the atmosphere) is increased by P.P.B. (5). Therefore, a diminished cardiac output alone could not reduce the blood flow in the finger unless accompanied by a peripheral vasoconstriction. Furthermore, the reduced cardiac output endures throughout P.P.B., whereas the skin-cooling effect is transient. If a marked transient diminution is ascribed to the left cardiac output as a result of the diminished venous return to the right heart, it must be noted that the hemodynamics probably readjust within a fraction of a minute to the stable enduring cardiac output which has been reported. In contrast the peak skin-cooling effect occurs within 2–3 minutes and may last 9–11 minutes.

The experiments reported in this paper offer further evidence for this vasomotor reflex, but they show in addition that the reflex is not initiated by the inflation of the chest which occurs in P.P.B. because it is not diminished when an inflated vest is worn to prevent abnormal chest expansion. (The use of a vest increased the skin cooling effect of P.P.B. in 12 of the total of 17 experiments; see fig. 2.) Furthermore, it occurs in negative pressure breathing as well as in positive pressure breathing. The reflex seems to involve the same mechanism which operates during deep inspiration and deep expiration, both of which are shown to be accompanied by a peripheral vasoconstriction.

In 1895 Binet and Sollier first observed a diminution of hand and cerebral volume following a deep inspiration (8). Uhlenbruck in 1924, as a result of his observations of arm and foot volumes, stated that all voluntary changes of respiration such as coughing, laughing, sighing, etc. produced peripheral vasoconstriction (9). Since then numerous observers have noted this phenomenon (10, 11, 15, 16). The work of Bolton *et al.* (10) and Mulinos and Shulman (11) seems to prove that this diminution in peripheral volume following a deep inspiration is a true reflex. Bolton demonstrated that it did not occur in the sympathectomized limb, and Mulinos and Shulman showed that it occurred distal to an arm cuff inflated to complete occlusion. Our own work demonstrates that reduced finger volume and skin-cooling occurs distal to a partially occluding arm cuff following not only deep inspiration but also deep expiration, indicating in both instances that the observed results are not directly due to pressure changes originating in the thorax, and transmitted through the veins.

This identical response to seemingly opposite causes is perplexing and makes interpretation difficult. However, consider the hemodynamics in each case, with regard to intrapleural pressure and its effect in venous return. It will be noted that P.P.B. and a deep expiration have the same effects in that both increase the intrapleural pressure and thereby diminish the venous return. Similarly negative pressure breathing and a deep inspiration can be related, in that both diminish the intrapleural pressure and consequently increase the venous return.

In the first case the dammed venous blood will stretch the extra thoracic veins, as demonstrated by Holt (4), Otis *et al.* (6) and Carr and Essex (17). In the second case the increased venous return must stretch the large intrathoracic caval veins and the right auricle.

In 1934 McDowall (12) demonstrated that stretching the right auricle produced peripheral vasoconstriction. In 1937 Nonidez (13) demonstrated histologically the presence of what he called stretch receptors in the intrapericardial portions of the inferior and superior vena cavae and pulmonary veins. It is therefore quite logical to assume that it is the stretching of the right auricle and the large intrathoracic veins that initiates the peripheral vasoconstriction incident to a deep inspiration and negative pressure breathing, since both of these maneuvers diminish the intrapleural pressure, thereby increasing the venous return to the heart and dilating the heart and veins.

If we postulate similar stretch receptors in the extrathoracic large veins, we might possibly explain the reflex peripheral vasoconstriction incident to a deep expiration and positive pressure breathing, since both of these raise the intrapleural pressure, thereby damming venous blood and causing the extrathoracic veins to stretch. It is in accord with this theory that the use of a vest with P.P.B., which increases the rise in intrapleural pressure (6, 14), thereby further impeding venous return and causing increased stretching of the large extra thoracic veins, likewise seems to increase the reflex skin cooling.

But this explanation appears paradoxical since it requires that as the central pressoreceptors relax, causing a relative arterial vasodilation, the peripheral pressoreceptors are stimulated, resulting in arterial vasoconstriction. However, there are certainly other instances where one reflex dominates over another; furthermore, evidence for the existence of pressoreceptors in the abdominal vena cava might be construed from the work of Erlanger and Gasser (18). When they partially occluded the inferior vena cava in dogs, thus damming blood back into the abdomen, they noted an increase in peripheral vasomotor tone as determined by rate of inflow into the femoral artery. It is possible that this vasoconstriction might have been reflexly initiated by stimulation of pressoreceptors in the abdominal vena cava.

Perhaps a simpler interpretation would be that the observed vasoconstriction in all cases represents an irradiation from the respiratory center to the vasomotor center as a result of rather strenuous voluntary or conscious changes in normal respiration comparable possibly to an alarm reaction. This latter hypothesis, however, fails to explain the increased reflex vasoconstriction when a pneumatic vest is worn, and it ignores the demonstrated presence of pressoreceptors in right auricle and large intrathoracic veins capable of producing the vasoconstriction observed with negative P.B. and deep inspiration. A more specific hypothesis is to be preferred.

SUMMARY

1. Further evidence from skin temperature and plethysmograph measurements is presented to show that positive pressure breathing results in a peripheral vasoconstriction.

2. This reflex does not depend upon an expansion of the chest, because it occurs equally well in negative pressure breathing and equally well or perhaps even better when expansion of the chest due to positive P.B. is prevented by the wearing of a pneumatic vest by the subject.

3. A similar reflex occurs after either a single deep rapid inspiration or a single deep rapid expiration.

4. It is thought likely that the vasomotor reflex incident to a deep inspiration and negative pressure originates from pressoreceptors in right auricle and intrathoracic large veins.

The author wishes to express his sincere gratitude to Professor Wallace O. Fenn for his patient instruction and constructive criticisms, without which this work would not have been possible. It also gives pleasure to acknowledge the aid of Dr. Elmer Tuttle in the construction of the finger plethysmograph, and the many well-received advices of Dr. Hermann Rahn and Dr. Arthur B. Otis.

REFERENCES

1. Fenn, W. O. and L. E. Chadwick. This Journal **151:** 270, 1947.
2. Fenn, W. O., A. B. Otis, H. Rahn and L. E. Chadwick. This Journal **151:** 258, 1947.
3. Bateman, J. B. and C. Sheard. J. Aviation Med. **17:** 568, 1947.
4. Holt, J. P. This Journal **134:** 292, 1942.
5. Dern, Raymond J. and W. O. Fenn. J. Clin. Invest. **28:** 3, 460–467, 1947.
6. Otis, A. B., H. Rahn, M. Brontman, L. J. Mullins and W. O. Fenn. J. Clin. Invest. **25:** 3, 413–421, 1946.
7. Richards, Jr., D. W. Federation Proc. **4:** 215, 1945.
8. Binet, A. and P. Sollier. Archives de physiol. Normale et Pathol., **7:** 719–734, 1895.
9. Uhlenbruck, P. Z. Biol., **80:** 317–341, 1924.
10. Bolton, B., E. A. Carmichael and G. Stürup. J. Physiol. **86:** 83, 1936.
11. Mulinos, M. E. and I. Shulman. This Journal **125:** 310–321, 2, 1939.
12. McDowall, R. J. Physiol. **81:** 5P, 1934.
13. Nonidez, J. F. Am. J. Anat. **61:** 203–231, 1937.
14. Otis, A. B., H. Rahn and W. O. Fenn. This Journal **146:** 307–317, 1946.
15. Soldati, L., E. A. Carbanne and A. S. Introzzi. Rev. Argent. Cardiol. **8:** 383, 1942.
16. Burch, G. E. Am. Heart J. **33:** 48–75, 1947.
17. Carr, D. T. and H. E. Essex. Am. Heart J. **31:** 53–73, 1946.
18. Erlanger, J. and H. S. Gasser. This Journal **49:** 151–173, 1919.

PITUITARY-ADRENAL CORTICAL ANTAGONISM TO ESTROGENIC STIMULATION OF THE UTERUS OF THE OVARIECTOMIZED RAT[1]

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The earliest known manifestation of estrogenic stimulation in the rat appears to be the acute shift of water into uterine tissue (1). This response has been shown to lend itself to accurate measurement both in the immature rat (1) and in the mature ovariectomized animal (2, 3).

The demonstration that this initial uterine response to estrogen is depressed in the eviscerated (2) and in the partially-hepatectomized rat (3) has led to the concept that the liver may be involved directly or indirectly in the activation of exogenous estrogen. The possibility remained, however, that these stressful conditions might exert their inhibitory influence via the adrenal cortex. This possibility would seem to be particularly likely in view of the intimate relationship between adrenal cortical activity and salt and water metabolism.

The present investigations were carried out in an attempt to ascertain the rôle of the adrenal cortex in the early response of the uterus to estrogenic stimulation. The results obtained indicate that increased pituitary-adrenal cortical activity has an inhibitory effect upon this response. Decreased activity would appear to have the opposite effect. Certain profound stresses may accentuate pituitary-adrenal cortical inhibition of estrogenic activity. Partial hepatectomy appears to constitute a sufficient stress in this regard, but the evidence reveals that the depression of estrogenic response in the absence of the intact liver cannot be explained entirely on this basis.

MATERIALS AND METHODS

Most of the procedures employed have been described in detail in earlier reports (2–4). Young adult virgin rats of an inbred Sherman strain were used approximately two weeks after ovariectomy. During the experimental period the animals were in most instances kept in individual cages, were protected from extremes of temperature and were allowed free access to water and Purina laboratory chow. Intravenous solutions were administered via the saphenous vein in the amount of 0.5 ml. per 100 gram body weight. These included control saline, alpha-estradiol[3] in physiological saline (3), saline suspensions of progesterone[4], testosterone[3], Compound A[5] and desoxycorticosterone acetate[5], and in addition, adrenocorticotrophin[6], casein and lactalbumin suspended in the estrogen solutions. Alternatively, in some cases adrenocorticotrophin in saline was administered intraperitoneally. Adrenal cortical extract in oil (Upjohn) was injected subcutaneously.

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[3] Crystalline alpha-estradiol and testosterone were obtained through the courtesy of Dr. E. Schwenk of the Schering Corporation.

In all these studies, the uteri were removed four hours after the administration of estrogen and weighed in both the wet and dry state. The maximal uterine water imbibition response to intravenously administered alpha-estradiol has been found to occur at this time in the control ovariectomized rat (5).

RESULTS

1. THE INFLUENCE OF ADRENALECTOMY ON THE UTERINE RESPONSE TO ESTROGEN. a. *Immediate effect.* The uterine response of the castrate rat to alpha-estradiol administered immediately after adrenalectomy was found not to be significantly different from that observed in unoperated animals. This is shown for two doses of estrogen in table 1. The lower dosage (0.5 microgram per 100 grams body weight) was apparently maximal under these conditions since double the quantity had no additional effect.

It may be noted that animals injected immediately after lumbar sham operation (see Group 4, table 5) responded in a fashion similar to that observed in the unoperated animals, and in animals adrenalectomized immediately prior to estrogen administration. All values in the tables are expressed as the mean ± the standard deviation, followed by the range in parentheses.

b. *Delayed effect.* The response to estrogen in adrenalectomized rats two days after operation was strikingly different from that observed in similar animals immediately after adrenalectomy (table 5). It is apparent that a significant enhancement in uterine water imbibition occurred in the adrenalectomized group (48 hours) over the values found in a control series of lumbar shams injected immediately after operation (0 hours). The other results tabulated in table 5 will be discussed below (section 4 b).

In certain groups of animals adrenalectomized 48 hours prior to estrogen administration, 0.9 per cent saline containing 5 per cent glucose was given as the drinking solution up to the morning of the experiments, at which time 0.9 per cent saline was substituted in a manner similar to that described by Venning *et al.* (6). This procedure had no influence on the results.

2. THE PITUITARY CONTROL OF ADRENAL ANTAGONISM TO ESTROGEN. a. *Effect of adrenocorticotrophin.* The results reported above suggested the possibility that the uterine response to estrogen was normally inhibited by adrenal

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[4] Crystalline progesterone was generously provided by Dr. Robert P. Jacobson.

[5] Crystalline samples of Compound A and desoxycorticosterone acetate were kindly placed at the disposal of the investigators by Drs. A. White and C. N. H. Long.

[6] Adrenocorticotrophin was prepared from pig pituitary glands by Dr. F. Saunders of G. D. Searle & Company. Approximately 25 micrograms of this material, injected intraperitoneally into test rats, was capable of causing a significant depression in adrenal ascorbic acid and in blood lymphocyte level in one to two hours.

TABLE 1. IMMEDIATE EFFECT OF ADRENALECTOMY ON THE UTERINE RESPONSE TO ESTROGEN

GROUP	NO. OF RATS	WET WT. UTERUS	DRY WT. UTERUS	PERCENT UTERINE WATER
A. 0.5 microgram alpha-estradiol per 100 grams body wt.				
		mgm/100 gm. body wt.	*mgm/100 gm. body wt.*	
Unoperated.........	5	67.1 ±11.2 (40.2 to 81.7)	9.8 ±1.9 (6.4 to 11.7)	85.3 ±0.8 (84.1 to 86.2)
Adrenalectomized...	6	63.9 ±21.5 (42.7 to 110.0)	9.5 ±3.2 (6.2 to 15.4)	85.1 ±0.9 (83.4 to 86.0)
B. 1.0 microgram alpha-estradiol per 100 grams body wt.				
Unoperated.........	5	59.9 ±12.1 (45.3 to 78.6)	8.9 ±1.5 (7.1 to 11.0)	84.9 ±0.7 (84.1 to 85.9)
Adrenalectomized...	16	68.1 ±16.7 (34.2 to 95.3)	10.3 ±3.4 (5.4 to 13.6)	84.9 ±0.5 (84.0 to 85.7)

All animals were autopsied four hours after intravenous administration of estrogen.

TABLE 2. INFLUENCE OF ADRENOCORTICOTROPHIN ON ESTROGEN-INDUCED IMBIBITION OF WATER BY THE UTERUS

GROUP	NATURE OF PROTEIN	DOSE	ROUTE	NO. OF RATS	PER CENT UTERINE WATER	'p'[1] VALUE
A. 0.5 microgram alpha-estradiol per 100 grams body wt.						
		mgm./100 gm. body wt.				
Unoperated......	None	—	—	5	85.3 ±0.8 (84.1 to 86.2)	—
Unoperated......	ACTH	2.5 to 3.0	i.v.	11	80.6 ±1.0 (78.6 to 82.0)	<0.01
Adrenalectomized.	ACTH	2.5	i.v.	6	83.8 ±0.7 (82.7 to 84.4)	0.03
Unoperated......	Casein or lact.	2.5 to 3.0	i.v.	7	84.3 ±1.5 (82.3 to 87.0)	0.2
Unoperated......	ACTH	2.5	i.p.	6	81.8 ±2.0 (78.3 to 84.0)	<0.01
Unoperated......	ACTH	5.0	i.p.	8	80.1 ±0.7 (79.3 to 81.5)	<0.01
B. 1.0 microgram alpha-estradiol per 100 grams body wt.						
Lumbar sham.....	None	—	—	14	84.5 ±1.4 (81.0 to 86.6)	—
Lumbar sham.....	ACTH	2.5 to 5.0	i.v.	5	80.1 ±1.0 (79.0 to 81.9)	<0.01

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[1] Between the control (first) member in each of the two estrogen dosage groups and the remaining members of that group; calculated by the Fisher 't-test'.

TABLE 3. INFLUENCE OF CRYSTALLINE STEROIDS AND ADRENAL CORTICAL EXTRACT ON THE UTERINE WATER RESPONSE TO ESTROGEN[1]

TREATMENT[2]	DOSE (PER 100 GRAMS BODY WT.)	NO. OF RATS	PER CENT UTERINE WATER	'p'[3] VALUE
Control	—	16	85.1 ±0.7 (83.4 to 86.2)	—
Compound A	1.0 *mgm.*	6	84.8 ±0.8 (83.4 to 85.8)	0.5
DOCA	1.0 *mgm.*	6	83.8 ±1.3 (81.3 to 85.5)	0.01
Progesterone	1.0 *mgm.*	6	83.7 ±0.6 (82.5 to 84.6)	<0.01
Testosterone	1.0 *mgm.*	5	84.4 ±1.3 (82.8 to 86.6)	0.2
ACE	1.0 *ml.*	6	84.5 ±1.1 (83.3 to 85.8)	0.2

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[1] Alpha-estradiol (0.5 microgram per 100 grams body wt.) was injected intravenously into all animals four hours before autopsy.

[2] The crystalline steroids were administered intravenously in saline suspension immediately after estrogen injection; adrenal cortical extract in oil (Upjohn) was given subcutaneously two hours earlier. DOCA—Desoxycorticosterone acetate; ACE—adrenal cortical extract.

[3] Calculated by the Fisher 't-test' between the control group and each of the succeeding groups.

TABLE 4. AMELIORATING EFFECT OF ADRENALECTOMY ON THE INHIBITION OF THE UTERINE RESPONSE TO ESTROGEN IN THE PARTIALLY HEPATECTOMIZED RAT[1]

GROUP	NO. OF RATS	PER CENT UTERINE WATER	'p' VALUE[2]
Unoperated	16	85.1 ±0.7 (83.4 to 86.2)	—
Partially-Hepatectomized	9	80.1 ±0.8 (78.9 to 81.4)	<0.01
		<0.01[3]	
Adrenalectomized, Partially-Hepatectomized	10	82.8 ±1.3 (79.8 to 84.3)	<0.01

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[1] Alpha-estradiol (0.5 microgram per 100 grams body wt.) was injected into all animals four hours before autopsy, and, in the operated groups, immediately after hepatectomy.

[2] Between the unoperated group and each of the other two groups.

[3] The 'p' value calculated between the two operated groups as indicated.

secretion and that the enhancement in response observed 48 hours after adrenalectomy was a manifestation of adrenal hormone depletion. Table 2 and figure 1 show the results obtained when adrenal cortical secretion was increased by the injection of pituitary adrenocorticotrophic hormone.

Injection of adrenocorticotrophin simultaneously with, or immediately preceding, the administration of estrogen resulted in a marked reduction in the uterine response. In figure 1 the reduction in uterine wet and dry weights is shown, and in table 2 are indicated the reduction in per cent uterine water and the significance of this reduction. Adrenocorticotrophin (ACTH) given intravenously was found to produce a more effective inhibition than the same dose given intraperitoneally (2.5 to 3.0 mgm. per 100 gram body weight).

TABLE 5. ENHANCEMENT IN UTERINE RESPONSE TO ESTROGEN ADMINISTERED 48 HOURS AFTER ADRENALECTOMY AND OTHER OPERATIVE PROCEDURES[1]

GROUP	HOURS AFTER OPER.	NO. OF RATS	WET WT. UTERUS	DRY WT. UTERUS	UTERINE WATER CONTENT	
					per cent	'p' value[2]
			(mgm./100 gm. body wt.)	*(mgm./100 grams body wt.)*		
Abdominal sham..	0	5	59.2 ±8.2 (42.6 to 67.0)	9.3 ±1.3 (7.0 to 10.8)	84.3 ±1.0 (83.2 to 85.5)	—
Abdominal sham..	48	9	90.3 ±15.8 (72.5 to 118.6)	12.7 ±1.8 (10.3 to 15.4)	85.8 ±0.7 (84.6 to 87.0)	<0.02
Splenectomized...	48	8	82.4 ±16.0 (50.3 to 108.2)	11.6 ±2.5 (10.1 to 15.1)	86.0 ±0.7 (84.7 to 86.7)	<0.01
Lumbar sham.....	0	14	64.6 ±15.2 (42.3 to 96.2)	9.9 ±1.9 (7.0 to 14.0)	84.5 ±1.4 (81.0 to 86.6)	—
Lumbar sham.....	48	5	74.3 ±14.7 (50.4 to 86.4)	10.5 ±2.4 (6.7 to 12.7)	85.8 ±0.8 (84.5 to 86.7)	<0.05
Adrenalectomized.	48	8	78.9 ±17.8 (51.3 to 106.5)	10.6 ±2.5 (6.6 to 14.1)	86.5 ±0.3 (85.4 to 87.0)	<0.01

[1] Alpha-estradiol (1.0 microgram per 100 grams body wt.) was injected intravenously into all animals at the time indicated in the second column.

[2] Between the 48-hour uterine water values and those obtained in the corresponding zero-hour group.

The powerful inhibitory influence of adrenocorticotrophin on the uterine response to estrogen was abolished by removal of the adrenals (table 2 and figure 1). This would seem to indicate clearly that the pituitary-adrenal cortical system is involved in this relationship.

The behavior of normal and adrenalectomized rats injected with relatively large doses of ACTH was of considerable interest. The slow intravenous administration to animals with adrenals intact resulted in profound lassitude during the early part of the four-hour period following injection and preceding autopsy. On intraperitoneal administration of ACTH to similar animals recovery from the state of lassitude which ensued was more delayed. Adrenalectomized rats injected with this pituitary preparation did not exhibit this behavior. A general autopsy finding in most animals receiving adrenocorticotrophin was grossly visible adrenal engorgement.

b. *Administration of nonhormonal proteins.* The intravenous administration of lactalbumin or casein to animals with intact adrenals, under the same conditions as those under which ACTH was given, had no significant effect on the uterine response to estrogen (see table 2 and figure 1). Thus the specificity of the phenomenon of pituitary control over adrenal cortical inhibition of the latter response appeared to be established by these observations, in which *a*) the presence of intact adrenals was required for the effect of ACTH to be manifested and *b*) other protein materials could not duplicate the inhibitory effect of ACTH observed in the presence of the adrenal glands.

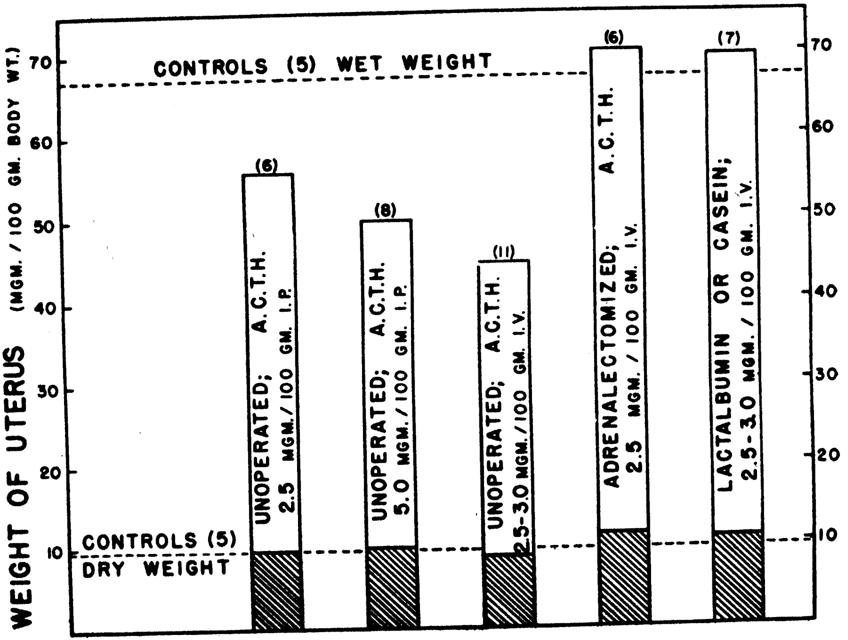

FIG. 1. INFLUENCE OF ADRENOCORTICOTROPHIN on the uterine response to intravenously administered alpha-estradiol. The total height of the bars represents the average uterine wet weight for each group; the cross-hatched portion, uterine dry weight. The dotted lines locate the corresponding average values obtained in control animals. The number of animals contributing to the average in each case is indicated by the number enclosed in parentheses. All animals were injected with 0.5 microgram of alpha-estradiol per 100 gram body weight in addition to the treatment described for each group in the figure. Autopsy was performed four hours later.

3. EFFECT OF CERTAIN CRYSTALLINE STEROIDS AND OF ADRENAL CORTICAL EXTRACT. In an effort to reproduce the observed adrenal antagonism to estrogen elicited by adrenocorticotrophin, crystalline steroids of adrenal origin (Compound

A and desoxycorticosterone acetate) were administered intravenously in saline suspension at a dose level of 1.0 mgm. per 100 gram body weight to castrate animals immediately following the intravenous injection of alpha-estradiol. In addition, two crystalline steroids, testosterone and progesterone, which are known to antagonize certain estrogenic effects, were included in the group of compounds studied. Finally, the effect of adrenal cortical extract in oil, given subcutaneously two hours prior to estrogen administration, was also investigated.

Inhibition of the uterine water response to estrogen was observed only in the animals to which desoxycorticosterone acetate and progesterone were administered (table 3). The depression observed with these compounds was not as profound as that elicited by stimulation of endogenous adrenal cortical secretion with ACTH. Factors of dosage, route and vehicle of administration, possible synergism of two or more hormones, etc. may have been involved in the failure to duplicate the effects of the pituitary hormone.

4. THE RELATIONSHIP OF PARTIAL HEPATECTOMY AND OTHER STRESSES TO THE PITUITARY-ADRENAL ANTAGONISM TO ESTROGENIC ACTIVITY. a. *Immediate effects: application of the stress simultaneously with estrogen administration.* The response of the ovariectomized rat to exogenous estrogen has been shown to be greatly diminished immediately following, and up to 24 hours after, partial hepatectomy (3). On the other hand, a variety of other traumatic procedures such as lumbar sham, abdominal sham and splenectomy (4) carried out immediately prior to estrogen administration had no significant depressing effect on the uterine response. In view of the potent action of ACTH in inhibiting the response to estrogen, and of the observations of others that many stresses are capable of activating the pituitary-adrenal cortical system (7, 8), it appeared necessary to re-examine the phenomenon of inhibition of uterine response to estrogen produced by partial hepatectomy. In addition, attempts were made to activate the pituitary-adrenal system by other stresses such as cold.

The relationship of partial hepatectomy and pituitary-adrenal antagonism to estrogenic stimulation of the uterus was studied in the following manner. One group of castrate rats was partially hepatectomized in the manner previously described (3); a second group was subjected to adrenalectomy in addition to removal of two thirds of the liver. Adrenalectomy was performed via the abdominal incision required for hepatectomy and immediately preceding the latter to prevent possible adrenal discharge concomitant with operative trauma. Estrogen was then administered to both groups in the usual manner (cf. table 4).

The immediate reduction in uterine response to estrogen, previously observed in the partially hepatectomized animal (3), was again apparent (average uterine water per cent 80.1 ± 0.8, compared to 85.1 ± 0.7 in the unoperated group). Adrenalectomy, carried out immediately prior to partial removal of the liver, had the effect of only partially preventing this decrease in sensitivity (average uterine water per cent of 82.8 ± 1.3). As shown by the table, the differences between all the groups are highly significant. It would appear, therefore, that only a portion of the inhibitory effect of partial hepatectomy on the uterine response to estrogen is mediated via the pituitary-adrenal cortical system.

The effect of cold stress was studied by placing a group of six ovariectomized rats in the cold room at 2 ± 2°C. in individual cages for one hour. At the end of this time, each animal was momentarily removed from the cold room, injected intravenously with 0.5 micrograms of alpha-estradiol per 100 grams and then returned to the cold room until the time of autopsy four hours later. The uterine water content in this group averaged 83.7 ± 0.8 per cent as compared with a control group of 16 animals with an average of 85.1 ± 0.7 per cent; the p value of this difference was less than 0.01.

b. *Delayed effects: application of the stress 48 hours before estrogen administration.* Stresses which appear incapable of causing inhibition of estrogenic activity may nevertheless activate the pituitary-adrenal system. Table 5 reveals that splenectomy, abdominal sham and lumbar sham, carried out 48 hours earlier, all resulted in an enhancement in estrogenic activity similar to that previously observed 48 hours after partial hepatectomy (3). Since an enhancement in the uterine response also occurs in animals which have been adrenalectomized two days earlier, it appears that some degree of adrenal exhaustion may supervene within 48 hours of other traumatic procedures, regardless of whether the stress was initially severe enough to cause a depression in uterine response.

DISCUSSION

The relationship between the adrenal cortex and the gonads has been studied in some detail by many investigators. Several workers have reported that pituitary extracts exhibiting adrenocorticotrophic activity, or extracts of the adrenal cortex itself, have a growth-promoting effect on the generative tract of the female rat under certain conditions (9–11). This has been assumed to be due to extra-ovarian sources of estrogen; this possibility has been favored in view of the isolation of estrone from beef adrenals by Beall (12).

In contrast to the above, some reports have indicated that suppression of growth of the female genital structures may occur following administration of adrenal cortical extracts to rats (13, 14). Asher and Klein (14) assumed this effect to be due to the presence of androgenic material. That other substances known to be present in the adrenal may have been responsible for the inhibition however, was indicated by the work of Robson (15), who found that the proliferative action of estrogens on the vaginal epithelium of the mouse can be antagonized by desoxycorticosterone and progesterone as well as by testosterone.

In the present investigation it has been found that at least the early manifestations of estrogenic stimulation of the uterus are normally under the damping influence of adrenal cortical secretion. Depression of circulating adrenal cortical hormones, such as occurs 48 hours after adrenalectomy, and presumably, such as may occur a similar length of time after traumatic procedures which stimulate adrenal hypersecretion, resulted in an enhancement in the uterine response to estrogen. Conversely, procedures which cause an excessive and rapid secretion of adrenal steroids resulted in a profound depression in estrogen sensitivity (adrenocorticotrophin administration, exposure to cold, partial hepatectomy).

The mechanism of action of the adrenal cortex in depressing the uterine response to estrogen is obscure at the present time. The dependence of this response upon shifts in water and electrolytes (16) suggests that the influence of the adrenal steroids on uterine sensitivity may be exerted via this mechanism. Long and Zuckerman (17), in fact, postulated that the periodic fluctuations in estrogenic sensitivity noted in castrate animals (18, 19) might be due to periodic changes in adrenal-controlled water metabolism. Progesterone (20), and desoxycorticosterone acetate are active in causing changes in salt and water metabolism, and among the steroids studied alone possessed any measure of antagonism to estrogenic stimulation of the uterus.

Alternatively, it is possible that the general phenomenon of estrogen-progesterone antagonism (cf. 21) may be responsible for the inhibitory influence of progesterone and desoxycorticosterone acetate on estrogenic stimulation of the uterus. If this be the case, the effectiveness of the latter substance may be due to its progestational activity (22), and the effects of both steroids would presumably be exerted directly on the uterus in some unknown fashion.

The previously observed effects of abdominal evisceration (2) and partial hepatectomy (3), producing a potent inhibition of estrogenic stimulation of the uterus, may now be regarded as being at least partially due to adrenal cortical hypersecretion. That the pituitary-adrenal cortical system is discharging at a very rapid rate in the eviscerated animal has recently been demonstrated by Vogt (23). It seems likely, however, that removal of the liver also deranges other functions which are involved in estrogenic activity (3). In support of the latter statement is the observation that removal of the adrenals just prior to partial hepatectomy alleviated but did not obliterate the depressing effect of liver removal on estrogenic sensitivity. Under similar circumstances, adrenalectomy in the otherwise intact castrate rat was capable of preventing the inhibitory influence of adrenocorticotrophin.

The early inhibitory effects of partial hepatectomy have been shown by Roberts and Szego (3) to be succeeded by a gradual return to normal estrogenic activity, followed by an enhancement in the response 24 to 48 hours after operation. This increase in sensitivity was observed to parallel the rate of liver regeneration in a striking manner, and it was postulated that the two phenomena were interrelated. Although this conclusion may have been warranted, it is apparent from the results reported above that at least a portion of this effect may be related to the concentration of adrenal cortical hormones (or of adrenocorticotrophin) in the body at various times after partial hepatectomy. Since various other traumatic procedures (splenectomy, sham operation) resulted in an enhancement in estrogenic activity 48 hours after operation which was similar to that observed in partially hepatectomized animals, it appears likely that adrenal hypersecretion observed immediately after partial removal of the liver is gradually succeeded by depletion of these hormones.

Whatever the exact nature of pituitary-adrenal cortical antagonism to estrogenic stimulation of the uterus, the implications are immediately apparent. Many conditions of stress are apparently capable of modifying estrogenic response in one direction or the other, depending on the degree of trauma and the elapsed time. The application of these phenomena to clinical and experimental studies remains to be elucidated.

SUMMARY

An antagonism has been demonstrated between pituitary-adrenal cortical activity and the sensitivity of the rat uterus to estrogenic stimulation. Adrenal cortical secretion normally appears to exert a damping effect on estrogenic activity, as indicated by an enhancement in this activity 48 hours after operative procedures which apparently cause a depletion of corticosteroids in the organism (e.g., adrenalectomy, partial hepatectomy, splenectomy, sham operation).

Hyperactivity of the pituitary-adrenal cortical system was shown to have an inhibitory effect on the uterine response to estrogen. This phenomenon appeared after the injection of pituitary adrenocorticotrophic hormone and after severe stress (exposure to cold, partial hepatectomy). Inhibition could also be produced by intravenous administration of suspensions of crystalline progesterone and desoxycorticosterone acetate, but not by similar solutions of testosterone and Compound A, or by subcutaneous administration of whole cortical extract in oil. The inhibition of estrogenic activity by adrenocorticotrophin in the presence of the adrenals could not be duplicated with nonhormonal proteins such as casein and lactalbumin. Bilateral adrenalectomy prevented the inhibitory effect of adrenocorticotrophin.

The powerful inhibition of estrogenic activity observed immediately after partial hepatectomy was not completely reversed by previous adrenalectomy. Apparently, the depressing influence of partial hepatectomy cannot be explained entirely on the basis of pituitary-adrenal stimulation and must be referred in part to the removal of functional hepatic tissue.

REFERENCES

1. Astwood, E. B. Endocrinology **23:** 25, 1938.
2. Szego, C. M. and S. Roberts. Endocrinology **36:** 104, 1945.
3. Roberts, S. and C. M. Szego. Endocrinology **40:** 73, 1947.
4. Szego, C. M. and S. Roberts. Endocrinology **41:** 269, 1947.
5. Roberts, S. and C. M. Szego. Unpublished observations.
6. Venning, E. H., V. E. Kazmin and J. C. Bell. Endocrinology **38:** 79, 1946.
7. Selye, H. J. Clin. Endocrinol. **6:** 117, 1946.
8 Long, C. N. H. Federation Proc. **6:** 461, 1947.
9. Nelson, W. O. Anat. Rec. Suppl. **81:** 97P, 1941.
10. Moon, H. D. Proc. Soc. Exp. Biol. & Med. **37:** 36, 1937.
11. Parkes, A. S. Physiol. Revs. **25:** 203, 1945.
12. Beall, D. Nature **144:** 76, 1939.
13. Müller, C. Endokrinologie **8:** 5, 1931.
14. Asher, L. and O. Klein. Klin. Wo-chschr. **10:** 1076, 1931.
15. Robson, J. M. J. Physiol. **96:** 21P, 1939.
16. Talbot, N. B., O. H. Lowry and E. B. Astwood. J. Biol. Chem. **132:** 1, 1940.
17. Long, C. N. H. and S. Zuckerman. Nature **139:** 1106, 1937.
18. Zuckerman, S. Proc. Roy. Soc. (London) B. **123:** 441, 1937.
19. Zuckerman, S. J. Physiol. **92:** 12P, 1938.
20. Gaunt, R. and H. W. Hays. Science **88:** 576, 1938.
21. Allen, E., F. L. Hisaw and W. U. Gardner In Allen, E. Sex and Internal Secretions, Baltimore, 1939.
22. Miescher, K., W. H. Fischer and E. Tschopp. Nature **142:** 435, 1938.
23. Vogt, M. J. Physiol. **102:** 341, 1943.

CARDIOVASCULAR RESPONSE TO POSTURE AND THE PROBLEM OF FAINTNESS AND SYNCOPE IN THE SEMI-STARVED INDIVIDUAL

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The association of fainting with hunger is universally recognized and episodes of fainting are commonly reported among famine victims, particularly when such persons are required to stand upright (1). Sensations of 'faintness' and 'lightheadedness' are common in starvation areas (2). It would seem reasonable to suggest that semi-starvation results in a failure in the postural adjustment of the circulation. An opportunity to examine this question was presented by the Minnesota Semi-starvation Experiment in which 32 'normal' young men were studied before, during and after six months of semi-starvation.

These men lost an average of 24 per cent of their body weight and developed the typical picture of European famine victims, i.e. bradycardia, edema, polyuria, weakness and depression. A brief description of the experiment has been published elsewhere (3) and further details are given in several other papers (4–7).

SUBJECTS, CONDITIONS AND METHODS

The subjects from 20 to 33 years of age and free of disease were volunteers from Civilian Public Service Camps. During the entire experiment, the men lived in the laboratory and followed a carefully controlled regimen of activity which required about 3490 calories per day to maintain a constant weight during the control period. The restricted diet was designed to reproduce known starvation diets in Western Europe during World War II and was fed at an average level of 1658 calories per day for a period of six months. The men were fed prescribed diets during the first 12 weeks of rehabilitation and then allowed to eat ad libitum until the 20th week when the rehabilitation observations to be reported here were made. Thirty-one men were studied during semi-starvation and for 12 weeks of rehabilitation; 12 of these men were also studied after 20 weeks of rehabilitation.

The cardiovascular effects of posture were studied by the use of a tilting table equipped with a foot board. The pulse rate was counted by a stethoscope at the heart apex and the blood pressure readings were obtained with a sphygmomanometer in the usual manner. Every effort was made to ensure standard conditions for each test. The men were made familiar with the tilt table by a dummy test the week before control observations were made. The tests were carried out in an air-conditioned room at 78°F. and 50 per cent relative humidity. The subjects were studied before breakfast and did not perform any appreciable exercise between getting out of bed and taking the test. The observers watched for and noted signs of sympathetic stimulation such as restlessness, sweating of palms and other abnormalities. As a result of the fact that the time available for carrying out the tilt-table tests was limited, the majority of men were tested in the tilted position (68°) for five minutes. Blood pressures and pulse rates were obtained every 30 seconds after tilting to 68°. These values were averaged to find the tilted blood pressure and pulse rate. A selected group of nine men was tested for 10 minutes in the tilted position.

Blood sugars were determined by the method of Folin (8) using cadmium hydroxide as the precipitating agent. Blood for sugar determinations was drawn in basal rest and at the end of a half-hour walk on a motor-driven treadmill at 3.5 miles per hour and 10 per cent grade.

Results of the tilt-table test. The blood pressures and pulse rates observed in the tilt-table tests during the six months of restricted diet are presented in table 1. The semi-starvation figures show the usual bradycardia and tendency toward hypotension associated with semi-starvation. It should be noted that the change in pulse and blood pressure due to tilting was not greatly affected by semi-starvation so that the 'score', calculated according to Crampton (9), shows only a small deterioration.

TABLE 1. PULSE RATES AND SYSTOLIC BLOOD PRESSURES BEFORE AND AFTER TILTING TO 68° ON THE TILT TABLE

Mean values, standard deviations, differences and the 'score' according to Crampton (9) for 31 subjects. The column heads refer to control (C), 12 (S12) and 24 weeks of semi-starvation (S24).

CONDITION	PULSE RATE BEATS PER MIN.			SYSTOLIC BLOOD PRESSURE, MM. HG		
	C	S12	S24	C	S12	S24
Horizontal..	55.4 ± 7.0	38.0 ± 5.3	37.7 ± 5.9	115.7 ±6.5	98.7 ±7.8	96.1 ±7.9
Tilted......	71.7 ±10.1	56.2 ±12.4	55.9 ±15.8	110.7 ±9.1	92.6 ±8.2	90.0 ±8.2
Difference..	+16.3 ± 6.9	+18.2 ± 9.6	+18.2 ± 6.4	−5.0 ±5.2	−6.1 ±6.3	−6.1 ±8.1
Score.......	45.0 ±16.9	38.9 ±23.1	39.5 ±21.6			

During tilting systolic blood pressures were frequently recorded in the range of 80 to 90 mm. of Hg; the two lowest readings in satisfactory tests averaged 76 to 77 mm. of Hg during tilting. As semi-starvation proceeded, the sounds of Korotkow in the tilted subject became more muffled and in many cases the transition between phases became more difficult to determine with accuracy. This was particularly true of the third and fourth phases. The determination of the systolic blood pressure was in question on 6 occasions after 12 weeks of semi-starvation (S12) and on 2 occasions after 24 weeks of semi-starvation (S24). The accuracy of the diastolic pressure measurement was questioned by the observers in more than one half of the cases at both S12 and S24. However in 10 men satisfactory diastolic pressures were obtained at each testing period. The pulse pressures for these men are presented in table 2 along with the pulse rates and systolic blood pressures.

The average horizontal pulse pressure dropped considerably during semi-starvation but the tilted pulse pressure dropped to an average of 22.9 mm. Hg. As a result of the restricted diet the pulse 'product' (rate times pulse pressure, 10) was reduced by more than 50 per cent in the horizontal position but this function was not affected to such a large degree in the tilted position. These results point to a markedly reduced cardiac output in horizontal as well as in the tilted position.

TABLE 2. EFFECT OF SIX MONTHS OF SEMI-STARVATION ON THE PULSE RATE, SYSTOLIC PRESSURE, DIASTOLIC PRESSURE, THE PULSE PRESSURE AND THE PULSE PRODUCT BEFORE AND AFTER TILTING TO 68° ON THE TILT TABLE, ALONG WITH THE DIFFERENCES IN THESE VARIABLES DUE TO TILTING

Mean values for the 10 men in whom satisfactory diastolic blood pressure readings were obtained.

CONDITION	PULSE RATE		SYST. PRESSURE		DIAST. PRESSURE		PULSE PRESSURE		PULSE PRODUCT	
	C	S24	C	S24	C	S24	C	S24	C	S24
Horizontal..	58.2	37.6	116.1	99.2	69.0	66.2	47.1	33.2	27.4	12.5
Tilted......	73.7	58.0	112.5	92.9	79.3	70.3	33.2	22.9	24.4	13.3
Difference..	+15.5	+20.6	−3.6	−6.3	+10.3	+4.1	−13.9	−10.3	−3.0	+0.8

TABLE 3. EFFECT OF 6 MONTHS OF SEMI-STARVATION AND 20 WEEKS OF REHABILITATION ON THE PULSE RATE AND SYSTOLIC BLOOD PRESSURE BEFORE AND AFTER TILTING TO 68° ON THE TILT TABLE ALONG WITH THE DIFFERENCES IN THESE VARIABLES AND THE 'SCORE' ACCORDING TO CRAMPTON

Mean values for 12 men

CONDITION	PULSE RATE BEATS/MIN.			SYSTOLIC BLOOD PRESSURE, MM. Hg		
	C	S24	R20	C	S24	R20
Horizontal..................	57.9	37.3	65.8	116.9	94.0	111.8
Tilted......................	74.3	57.2	82.9	112.6	88.5	110.9
Difference..................	+16.4	+19.9	+17.1	−4.3	−5.5	−0.9
'Score'.....................	45.4	38.3	55.4			

After 20 weeks of rehabilitation (R20) the tilt-table test was carried out on 12 subjects. The results are presented in table 3. The pulse rate response to tilting at R20 was equal to the control but the systolic blood pressure did not drop as far as the control; as a result the score according to Crampton was a little 'improved' over the control. Satisfactory diastolic pressures are available for these men for the C and R20 periods and the mean 'tilted' pulse pressures for these periods were 31.3 and 30.9 mm. of Hg, respectively. It should be pointed out that at this time (R20) these men had regained their original weight.

Fainting on the tilt-table. Four of the 31 men who completed the experiment fainted on the tilt-table during the control observations. Two of these men fainted during the 5-minute test while the other two were in the '10-minute' group and fainted after between 5 and 10 minutes of tilting. It should be mentioned that both of the '5-minute' fainters fainted twice during the control testing so that it may be presumed that their fainting was not due to a temporary condition.

The two fainters who were in the 5-minute group were moved into the 10-minute group after 12 weeks of semi-starvation (S12); all four of these men withstood 10 minutes of tilting without difficulty. The pulse rates and systolic blood pressures of these men at S12 are presented in figure 1 along with similar data for the seven men in the 10-minute group who did not faint in the control period. In general, the 'fainters' at S12 had a slightly higher blood pressure and a little lower pulse rate than the non-fainters. The tendency for the systolic blood pressure of the 'fainters' to decline toward the end of the tilting period did not appear to be any greater than that shown by the non-fainters during the control observations. During all the tilt-table testing at both S12 and S24 only one subject (*No. 11*) fainted and this man fainted during the S12 test at four minutes of tilting. The only fainter who was available for testing at the end of 20 weeks of rehabilitation was subject *No. 119* and it is important to note that this man fainted after five minutes of tilting when he was tested at R20 but did not faint at either S12 or S24.

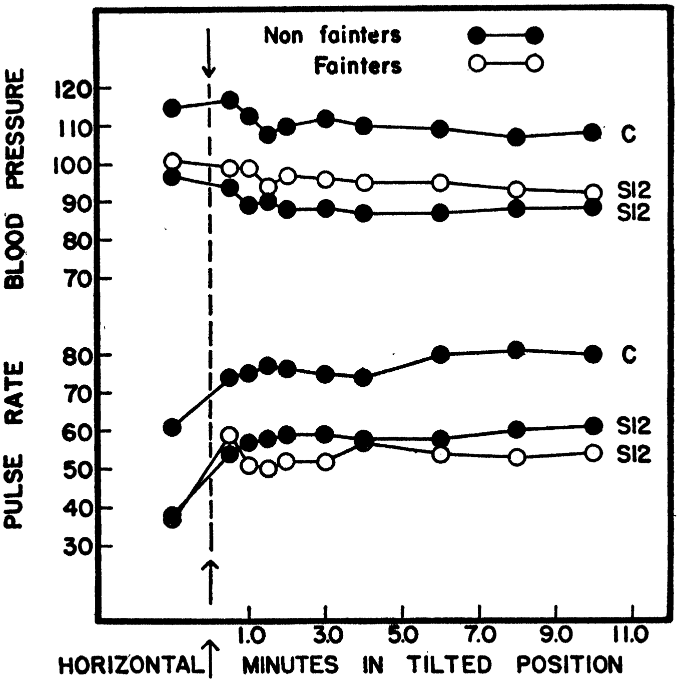

FIG. 1. AVERAGE PULSE RATES AND SYSTOLIC BLOOD PRESSURES of the seven men in the '10-minute group' ('non-fainters') before and after tilting during the control period and after 12 weeks of semi-starvation along with the average pulse rates and systolic blood pressures before and after tilting after 12 weeks of semi-starvation of the four men ('fainters') who fainted during the control period.

Blackouts, the sensation of faintness and spontaneous fainting. An almost universal complaint of the subjects in the Minnesota Experiment was some degree of faintness after standing up from a chair or bed. This was a very transitory phenomena which varied from a slight sensation of dizziness to a dimming of vision and 'blacking out' causing the subject to reach for a temporary support. No one ever lost consciousness during these episodes. Reports of 'dizziness' associated with change of posture were very numerous during the first six weeks of the restricted diet. They decreased after that time until there were very few complaints on this point during the last six weeks (S18 to S24). It was felt that the men learned to refrain from making a sudden transition to the vertical posture and in this way helped to avoid bringing on symptoms. It is interesting to note that complaints of dizziness associated with the assumption of the upright posture increased during the early part of rehabilitation when the men began to feel more vigorous; these complaints disappeared as appreciable weight gains were made.

It should be mentioned that these transient symptoms were not reproduced when the men were passively changed from the horizontal to the tilted position on the tilt table. The blood pressure readings 30 seconds after tilting showed no unusual change in semi-starvation as compared to the control period (see figure 1). Obviously, there was a difference between the effects of active and passive change of posture.

A number of men reported sensations of 'faintness', 'lightheadedness', 'dizziness' and 'weakness' which were not associated with postural change. These symptoms tended to occur near the end of the starvation period while the men were on their feet doing chores. In addition, a few men reported 'dizziness' on waking up in the morning which disappeared after getting out of bed and moving around a little. An attempt was made to test the response of the cardiovascular system to posture during one of these periods of faintness. This was possible with subject *No. 12* who was tested on the tilt table after doing chemical laboratory chores for two hours. He stated that for 30 minutes before the test he had been feeling 'weak' and 'faint'. He was tilted for 15 minutes and showed a satisfactory reasponse which consisted of an average tilted blood pressure of 86 mm. Hg, an average tilted pulse rate of 58 and no signs of sympathetic stimulation.

Only one clear episode of spontaneous syncope occurred during the course of the experiment. Subject *No. 11* fainted and remained unconscious for two or three minutes while waiting in line for his supper to be served. He recovered spontaneously and was able to finish his meal and return to the dormitory. This occurred after nine weeks of semi-starvation and it should be noted that the subject had a severe cold and had been given one-half grain of codeine and one-half grain of papaverine. Three weeks later this man fainted after four minutes of tilting on the tilt table. This subject was the only man to faint on the tilt table during all of the semi-starvation period.

Blood sugar. Mean blood sugar levels in work and rest, together with the standard deviations, are presented in table 4. Basal blood sugars are not available for these men during the control period but the extent of the deviation from normal may be judged by the values during rehabilitation; normal basal values in other young men in this laboratory have a mean of 72.0 mgm. per 100 ml. of blood. The changes in the basal blood sugars were certainly not large. The lowest recorded value was 55.6 and there were only two values between 56 and 60 mgm. per 100 ml. of blood. In terms of actual change, the blood sugar concentration of three subjects increased during rehabilitation 18 to 20 mgm. per 100 ml. of blood and three additional subjects made gains of 15 to 17 mgm. per 100 ml. of blood. On the other hand, much lower individual values for blood sugar were observed during work. There was one isolated case of a blood sugar during work of only 30 mgm. per 100 ml. of blood. There were five instances of work blood sugars at S24 in which the values fell within the range of 46 to 50 and 8 values within the range of 51 to 55 mgm. per 100 ml. of blood.

Symptoms which have been ascribed (11) to hypoglycemia were not numerous, did not occur with frequency in any individuals and were certainly not specific. In addition to 'weakness', 'faintness', 'lightheadedness' and 'dizziness', numbness and tingling in extremities, in situations where circulatory occlusion would not be expected, were reported on one or two occasions and one man reported an episode of trembling after walking on the treadmill.

TABLE 4. Blood sugar (mgm. per 100 ml.) in the basal state and in work. The 'basal' bloods were drawn before the men arose in the morning and the 'work' bloods were taken 25 minutes from the beginning of a 30-minute walk on the treadmill at 3.5 miles per hour and a 10 % grade

Means and standard deviations for 32 men

CONDITION	CONTROL	S12	S24	R12
Basal	—	—	63.4 ±4.4	73.1 ±3.7
Work	71.4 ±5.6	61.7 ±6.8	59.8 ±6.0	—

DISCUSSION

The observations reported here apply strictly to young men who have lost 24 per cent of their body weight during a six-month period on a restricted Western European diet. Difference in diet, in rate and amount of weight loss might well produce an entirely different picture; it might be expected that age and sex differences would also be of some importance.

There was no objective evidence that the 'blackouts' and 'faintness' which occurred when the men assumed verticality were due to cardiovascular failure. It has been shown in normal young men (12) that the rapid phase of adaptation to the vertical posture is complete in 30 seconds and blood pressure measurements during this time would have been necessary to check this point. Since it has been demonstrated (13) that 'blacking out' during positive acceleration is due to an inadequate cerebral blood pressure, it appears very likely that the 'blackouts' were the result of temporary circulatory failure during the rapid phase of adjustment (the first 30 seconds) to the vertical posture. The failure of the blackouts to be reproduced on the tilt table is surprising. However, some slight excitement was inevitably attendant on tilting the table and this factor may have accounted for the absence of transitory blackouts and faintness during the first 10 or 20 seconds of tilting.

On the other hand, it is evident that at least some of the complaints of 'light headedness', 'dizziness', etc., which were not associated with a sudden change in posture, cannot be ascribed to central circulatory incompetence. In this connection, it has been shown that sensations of faintness can occur in normal individuals in the upright posture without any decrease in the cardiac output and with adequate blood pressure (14). Leyton (2) ascribed most of the complaints of this nature which were noted in German prisoners of war to the reduced level of the blood sugar. Comparison with blood sugar levels which produce hypoglycemic symptoms in the normal individual are probably not valid since it is known that the nutritional state has a definite influence on the appearance of symptoms in relation to the fall in the blood sugar (15). However, in acute starvation with hard work (16) blood sugar levels between 30 and 50 mgm. per 100 ml. of blood have been observed in a number of men whose only complaint was a deadening fatigue. Finally, complaints of 'dizziness', 'faintness', etc. are extremely common in psychoneurosis and it seems possible that these complaints are merely part of the general picture of semi-starvation neurasthenia which existed to some degree in all of the men and has been described elsewhere (4).

It seems clear that the response of the 'fainter group' to tilting on the tilt board improved as these men lost weight. The following facts support this contention. The highly standardized conditions under which the tests were carried out resulted in reproducible fainting during the control period. Subject *No. 119* provided a good control experiment after he had regained his weight by fainting at approximately the same time of tilting which resulted in a faint during the control period. Finally, the 'five-minute fainters' withstood a tilting period during semi-starvation which was over twice as long as was necessary to produce faints consistently during the control period. A complete explanation of this improvement in the response to tilting during semi-starvation is not possible. However, it should be pointed out that the blood volume in cc. per kgm. increased to 120 per cent of the normal figure (5) and it is quite possible that this relative plethora would counteract pooling and filtration in the abdomen and extremities.

The pulse rate and blood pressure responses to the tilting position would appear to indicate a normal circulation in the vertical posture. This is particularly true if the 'scores' according to Crampton are used. However, evidence has been presented elsewhere (6) that during quiet standing the circulatory factor of safety as measured by the ratio of unused venous oxygen returned to the heart per minute to the metabolic rate was reduced by 50 per cent in the subjects of this experiment at the end of the semi-starvation period.

It is apparent then, that while the cerebral circulation is adequately maintained in the upright posture, any circulatory stress in addition to that of verticality might well result in failure of the cerebral circulation and syncope. It is perhaps not a coincidence that on the one occasion during the Minnesota Experiment when spontaneous syncope occurred, the subject was suffering from a severe cold, a disease which is frequently mentioned in the literature as a cause of poor cardiovascular adjustment to the vertical posture (17).

Among stresses which might well result in syncope in the starved individual 'queuing up', we may mention hot weather, dehydration as the result of diarrhea and respiratory infections.

SUMMARY

1. Thirty-one men who underwent six months of semi-starvation, during which time they lost 24 per cent of their body weight and developed the classical picture of starvation, were studied on the tilt table before and after 12 and 24 weeks of the restricted diet. Twelve of these men were studied after 20 weeks of rehabilitation.

2. Blood pressures and pulse rates were recorded in all 31 men for 5 minutes of the tilted position and for 10 minutes in a selected group of men. During semi-starvation the pulse rate and blood pressure were reduced to almost the same extent in both the tilted and horizontal position and the differences in these variables due to tilting remained essentially constant. On the other hand, the pulse pressure was markedly reduced in both positions.

3. After 20 weeks of rehabilitation the men had regained their original weight and at this time the response to tilting, as judged by pulse rate and blood pressure values, was a little better than in the control period.

4. In the control period two men fainted on two occasions each before 5 minutes of tilting. Both of these men were able to withstand 10 minutes of tilting during semi-starvation. Two additional men fainted between 5 and 10 minutes of tilting in the control period and both of these men withstood 10 minutes of tilting on two occasions during semi-starvation. The only one of these four men available for testing after recovery of his body weight fainted after four minutes of tilting.

5. 'Blackouts' and 'light headedness' on sudden rising were a universal complaint. Sensations of 'faintness' and 'dizziness' were occasionally reported which were not associated with postural changes. Only one real instance of spontaneous syncope occurred during semi-starvation.

6. Blood sugars were decreased an average of 10 mgm. per 100 ml. of blood in rest and 12 mgm. per 100 ml. of blood after walking one-half hour at 3.5 miles per hour on a 10 per cent grade.

7. These phenomena and their possible explanation are discussed.

REFERENCES

1. Zimmer, R., J. Weill and M. Dubois. New England Jour. Med. **230**, 303, 1944.
2. Leyton, G. B. Lancet **2**, 73, 1946.
3. Keys, A. J. Am. Diet. Assoc. **22**, 582, 1946.
4. Franklin, J. C., B. C. Schiele, J. Brozek and A. Keys. J. Clin. Psych. In press.
5. Henschel, A., O. Mickelsen, H. L. Taylor and A. Keys. This Journal **150**, 170 1947.

6. Keys, A., A. Henschel and H. L. Taylor. This Journal **150**, 153, 1947.
7. Keys, A., H. L. Taylor, O. Mickelsen and A. Henschel. Science **103**, 669, 1946.
8. Folin, O. J. Biol. Chem. **82**, 83, 1929.
9. Crampton, C. W. American Journal of Medical Science **160**, 721, 1920.
10. Schneider, E. C. and C. B. Crampton. This Journal **110**, 14, 1934.
11. Collens, W. S. and L. C. Boas. The Modern Treatment of Diabetes Mellitus. Charles C Thomas, 1946. Pp. 138–139.
12. Wald, H., N. Guernsey and F. H. Scott. Am. Heart Jour. **14**, 1, 1937.
13. Lambert, E. H. and E. H. Wood. Federation Proc. **5**, 59, 1946.
14. Starr, I. and A. J. Rawson. This Journal **134**, 403, 1941.
15. Jensen, H. F. Insulin, Its Chemistry and Physiology. The Commonwealth Fund: 80, 1938.
16. Taylor, H. L., J. Brozek, A. Henschel, O. Mickelsen and A. Keys. This Journal **143**, 148, 1945.
17. Berry, M. R., Jr., B. T. Horton and A. R. MacLean. Med. Clin. of North Am. **24**, 1095, 1946.

PHYSIOLOGICAL MECHANISM OF EXPERIMENTAL GOITROGENESIS

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Advent of chemical goitrogens that prevent the formation of thyroxine has revolutionized the study of thyroid physiology. Though the mechanism of action of all goitrogens has not yet been elucidated, it is definite that not all goitrogens act in the same way. By a method of study analogous to the elucidation of the various steps in tissue redox systems and by the use of enzyme inhibitors which act specifically on a certain enzyme component, it has been possible to differentiate thyroid physiology with respect to thyroxine formation into different stages by the use of different goitrogens, each of which acts specifically on a certain stage, although some goitrogens affect more than one stage.

The first step in thyroxine formation consists in the iodide concentration mechanism of the thyroid, though the anterior pituitary gland can also take uplarge amounts of iodine (1). This appears to be a complex ionic effect. Iodide and thiocyanate ions are neighbors in Pauli's system (2, 3) or in Hofmeister's series and so compete for the same systems in the body (4). As the isoelectric point of thyroid protein is near pH 5.1 under body conditions, it must behave as an anion and therefore cannot trap much iodide, so there must be some nonprotein mechanism in the thyroid for trapping a large part of iodide (5). Thiocyanate produces goiter by interfering with the iodide concentrating mechanism of the thyroid gland (4, 6–8), and the thiocyanate-goiter is prevented by feeding iodide (9). This is *one type* of goitrogen.

The second step in thyroxine formation consists in the oxidation of iodide ions into free iodine which reacts with tyrosine to form diiodotyrosine. Oxidation is produced by tissue redox systems, as the cytochrome-cytochrome oxidase or the peroxidase system. Some goitrogens prevent the action of the cytochrome-cytochrome oxidase system, as carbon monoxide, azides, sulfides, arsenic, etc.; some inhibit the peroxidase activity as thiourea, thiouracil, etc. (11); some compete with tyrosine for free iodine as p-aminobenzoic acid, sulfanilamides and other aniline derivative goitrogens (10). This is the *second type* of goitrogen.

The third step in thyroxine formation is the oxidation of two diiodotyrosine molecules to thyroxine through some enzyme systems. Agents which compete with diiodotyrosine for the enzyme systems which convert this to thyroxine also produce goiter, as do the various aniline derivatives consisting of p-aminobenzoic acid, sulfanilamides and thiouracils (12). This is a *third type* of goitrogen.

It should be noted that some goitrogens act in more than one step. An at-

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tempt has been made in the present investigation to elucidate some of the mechanisms of goitrogenesis with respect to iodide antagonism, tyrosine competition and to the higher potency of thiouracils.

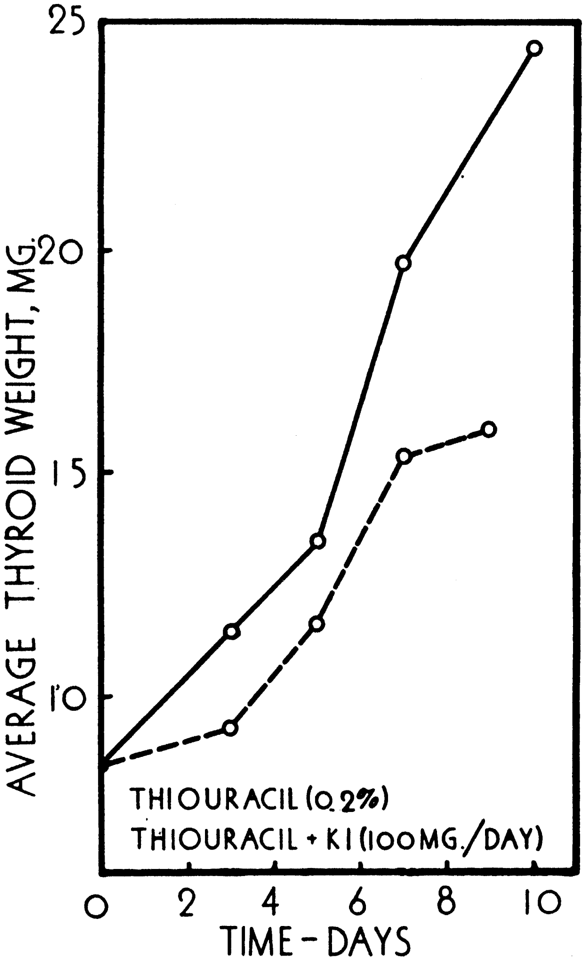

FIG. 1. THE EFFECT OF FEEDING POTASSIUM IODIDE 100 mgm. daily to rats fed 0.1 per cent thiouracil in the feed. Note the marked reduction in thyroid size in mgm. per 100 gram body weight.

EXPERIMENTAL

Experiments with potassium iodide. Ninety 100-gram albino rats were used; 10 of these were used as controls and the remaining 80 were placed on a diet containing 0.2 per cent thiouracil. One half of the thiouracil-fed rats were also fed potassium iodide (100 mgm. per day). On the third, fifth, seventh and ninth day of the experiment 10 rats from each group were sacrificed and their thyroids, adrenals and pituitaries weighed. The results on the thyroids are presented in figure 1. The average thyroid weights given are per 100 gram body weight of rat. Thyroid weight in the iodide-fed group was considerably less than in the control group. Larger doses of potassium iodide produced symptoms of iodism.

Experiments with tyrosine and glycine. Experiments were conducted on 56 albino rats divided into groups of 8 controls and two groups of 24, one given a diet containing 0.5 per cent p-aminobenzoic acid and the other, 0.3 per cent sulfadiazine. Each group of 24 rats was subdivided into 3 subgroups of 8 each. One of the subgroups served as drug control, one was fed 10 per cent tyrosine in the feed and the third subgroup was fed 10 per cent glycine in the feed.

The animals were killed after 18 days. The weight of the thyroids and adrenals are shown in table 1. Feeding 0.5 per cent p-aminobenzoic acid had only a very slight goitrogenic effect, while feeding tyrosine or glycine had no significant effect. The rats fed 0.3 per cent sulfadiazine showed a pronounced goitrogenic effect; the thyroid weight increased 100 per cent over that of the controls. Both body and thyroid weights were increased in those rats fed glycine. The feeding of tyrosine, on the other hand, produced a smaller thyroid, which is statistically significant from that of the rats fed glycine and sulfadiazine.

TABLE 1. INFLUENCE OF TYROSINE AND GLYCINE FEEDING FOR 18 DAYS; THE EFFECT OF SOME GOITROGENS WITH RESPECT TO THE WEIGHT OF BODY, THYROID, ADRENAL AND PITUITARY OF RATS

EXPERIMENT	N	PAB	PABT	PABG	SDG	SDT	SD
Initial body wt. grams	90	91	91	91	91	91	91
Final body wt. grams	125	123	121	143	149	134	136
Ratio thyroid wt. mgm. to 100 grams b. w.	7.71	8.41	8.69	8.35	17.90	13.87	15.79
Ratio adrenal wt. mgm. to 100 b. w.	16.99	17.40	18.49	14.65	14.57	17.23	15.85
Ratio pituitary wt. mgm. to 100 grams b. w.	4.32	4.71	4.68	4.19	4.50	4.78	5.22

N = normal control; PAB = 0.5% p-aminobenzoic acid; T = 10% tyrosine in feed; G = 10% glycine in feed; SD = 0.3% sulfadiazine

Experiments with thiouracil, tyrosine, glycine and thyrosine. In *experiment 1*, 50 male rats were used, 40 of these being placed on a diet containing 0.1 per cent thiouracil. Half of these had 10 per cent tyrosine added to their diet. Ten rats from each of the thiouracil controls and thiouracil-tyrosine-fed group were injected with 3 mcg. dl-thyroxine daily. After three weeks their thyroids and adrenals were weighed with the results presented in table 2.

In *experiment 2*, 50 female rats were used. Ten rats served as controls and 40 were fed 0.1 per cent thiouracil. Of these thiouracil-fed rats, 10 served as thiouracil controls and 10 were injected daily with 5 mcg. dl-thyroxine. The remaining 20 were also fed 10 per cent tyrosine in the feed, and ten of these were injected daily with dl-thyroxine. The resulting thyroid and adrenal weights are presented in table 3.

In *experiment 3*, 30 female rats were used. Ten served as normal controls; 20 were fed 0.1 per cent thiouracil and injected with 4 mcg. dl-thyroxine daily. Half of these were also fed 10 per cent glycine in the diet. The results are shown in table 3.

It will be seen from table 2 that tyrosine feeding lowers the thyroid weight in rats fed thiouracil. This result is similar to that obtained by feeding sulfadiazine. However, when a limited amount of thyroxine is daily supplied to the organism feeding tyrosine tends to increase the thyroid weight. *Experiment 2* in table 3 shows essentially the same result in female rats—tyrosine feeding lowering the thyroid weight in thiouracil-fed rats and increasing the thyroid weight when a limited amount of thyroxine has been injected daily. *Experiment 3* in table 3

TABLE 2. *Experiment 1.* A 3-WEEK EXPERIMENT ON MALE ALBINO RATS TO DEMONSTRATE THE INFLUENCE OF TYROSINE ON THE GOITROGENIC ACTION OF THIOURACIL

	N	Th	ThTy	ThThy 3 mcg.	ThThy 3 mcg Ty
Initial body weight grams	92	100	99	90	95
Final body weight grams	180	155	142	156	161
Ratio thyroid wt. mgm. to 100 grams body weight	7.41	31.01	27.87	15.54	16.91
Ratio adrenal wt. mgm. to 100 grams body weight	11.62	12.08	12.94	10.05	10.98

N = normal control; Th = 0.1 % thiouracil; Ty = 10% tyrosine in feed; Thy = dl-thyroxine injected.

TABLE 3. *Experiment 2 and 3.* EXPERIMENTS ON FEMALE ALBINO RATS TO DEMONSTRATE THE INFLUENCE OF TYROSINE WHEN THE ONLY SUPPLY OF THYROXINE TO THE BODY IS A LIMITED AMOUNT OF EXTRINSIC THYROXINE

	EXPERIMENT 2					EXPERIMENT 3		
	N	Th	ThTy	ThThy 5 mcg.	ThTyThy 5 mcg.	N	ThThy 4 mcg.	ThGThy 4 mcg.
Initial body wt. grams	73	74	75	74	75	201	196	194
Final body wt. grams	106	102	102	111	111	208	206	204
Ratio thyroid wt. mgm. to 100 grams body wt.	9.86	45.24	43.12	7.10	8.56	7.47	12.27	9.87
Ratio adrenal wt. mgm. to 100 grams body wt.	26.40	16.47	16.61	17.96	18.88	12.59	12.24	13.21

N = normal control; Th = 0.1% thiouracil; Ty = 10% tyrosine in feed; Thy = dl-thyroxine injected; G = 10% glycine in feed.

shows the effect of glycine with a limited supply of thyroxine daily. Thyroid weight is slightly decreased by feeding glycine under these conditions; this weight is significantly different from the thyroid weight obtained by feeding tyrosine under similar circumstances.

Experiments with uracil and thiouracil. Three groups of 8 albino rats each were used, one group serving as control. The remaining two groups were fed 0.1 per cent thiouracil, one group being fed, in addition to the thiouracil, 0.1 per cent uracil. After 18 days these were sacrificed, and the results are shown in table 4. It will be noted that uracil-fed rats have a lower thyroid and a higher adrenal weight than those fed thiouracil alone. The difference in thyroid weight between these two groups is statistically significant, the F value being 23.

TABLE 4. INFLUENCE OF URACIL ON THE GOITROGENIC EFFECT OF THIOURACIL

DRUG	GROUP	NO. OF RATS	AV. BODY WT. GRAM		THYROID WT. MGM. PER 100 GRAM B.WT.	ADRENAL WT. MGM. PER 100 GRAM B.WT.	PITUITARY WT. MGM. PER 100 GRAM B.WT.
			Initial	Final			
None	1	8	90	125	7.71	16.99	4.32
Thiouracil 0.1%	2	8	91	141	19.54	10.57	5.55
Uracil 0.1%	3	8	91	147	15.15	12.56	4.43

DISCUSSION

It has been postulated that thiopyrimidines act as goitrogens by reacting with several equivalents of iodine (11). Antagonism has been recently reported (13) between thiouracil and iodine, although such demonstration is difficult to show as the large amount of iodine required to demonstrate this action is likely to lead to iodism. Results shown in figure 1 in this paper demonstrate definitely that iodide antagonizes the goitrogenic effect of thiouracil. This supports the hypothesis that thiopyrimidines produce goiter by reacting with iodine, which is thereby made unavailable to iodinate tyrosine (11). This, however, is not the sole mechanism, as shown by feeding diiodotyrosine to thiouracil-treated animals when the goiter is still produced.

The aniline derivatives which are goitrogenic were assumed to compete with diiodotyrosine for the enzyme systems responsible for the conversion of diiodotyrosine to thyroxine (12), but as these compounds inhibited synthesis of diiodotyrosine from inorganic iodide, the hypothesis of competition was found to be unsatisfactory (14). It will be noted, on further analysis, that aniline derivatives may compete with tyrosine as well as with diiodotyrosine for iodine (10). It was, therefore, found desirable to feed tyrosine and observe the effect on the goitrogenicity of aniline derivatives. It will be seen from table 1 that tyrosine decreases the goitrogenic effect of sulfadiazine, the effect being especially marked when comparison is made with glycine-fed animals. No significant effect was noted with p-aminobenzoic acid, as the total goitrogenic power was very slight. A similar effect in case of thiouracil will also be noted in tables 2 and 3. The thyroid weight was lower when 10 per cent tyrosine was added to the diet in both male and female thiouracil-fed rats. The goitrogenic effect of thiouracil is so great that tyrosine can hardly react with any iodine which is quickly reacted upon by thiopyrimidines. But in the presence of tyrosine excess, as reported in this paper, some iodination of tyrosine does take place and presumably some synthesis of thyroxine. But as thiouracil also antagonized the oxidation of diiodotyrosine to thyroxine, the effect is not marked. Nevertheless, this experiment demonstrates that thiouracil, sulfadiazine and presumably other aniline derivatives compete with tyrosine for iodine in vivo. This is, however, not the sole mechanism, and these probably compete also with diiodotyrosine for the enzyme system responsible for its conversion into thyroxine.

A curious effect is shown by the data in tables 2 and 3. When the intrinsic source of thyroxine has been cut down by feeding thiouracil, and when only a limited amount of thyroxine is supplied, tyrosine has a goitrogenic effect (15, 16), while glycine helps the action of thyroxine. This seems to indicate that normally tyrosine competes with thyroxine in the tissues (15, 16).

The high goitrogenic effectiveness of the thiopyrimidines is very impressive. It has been postulated that thiouracil may compete for iodine with uracil, which is a component of ribonucleic acid (17). This was actually tested and the results are shown in table 4. Uracil was found to reduce the goitrogenicity of thiouracil. It is probable that uracil may act as a catalyst in iodination of tyrosine. This may explain why thiouracils are so very effective as goitrogens. It is thus concluded that *thiouracils function as goitrogen by reacting with iodine, inhibiting peroxidase activity, competing with tyrosine for iodination and with diiodotyrosine for oxidation and also competing with uracil in the body.*

SUMMARY

1. Thiouracil reacts with iodine and the goiter produced by its administration can be reduced by feeding large doses of potassium iodide. Complete inhibition of thiouracil is difficult due to the supervention of iodism.

2. Thiouracil competes for iodine with uracil, which is a component of ribonucleic acid. This explains the high effectiveness of thiouracils as compared to other chemical goitrogens.

3. Aniline derivatives produce goiter partly by competing with tyrosine for the available iodine in the body. They probably also compete with diiodotyrosine for the enzyme system necessary for its conversion to thyroxine.

4. Goitrogens have been classified into three categories with several subclasses. Attention has been drawn to some of the less known metabolic reactions of tyrosine, such as the thyroxine antagonism effect of tyrosine, which can be demonstrated best under conditions of limited supply of extrinsic thyroxine.

REFERENCES

1. McClendon, J. F. and W. C. Foster. Federation Proc. **6**: 275, 1947.
2. Smith, J. H. Virginia Med. Monthly **71**: 301, 1944.
3. Pauli, W. Münch. med. Wochschr. **50**: 153, 1903.
4. Baumann, E. J. and N. Metzger. Federation Proc. **6**: 237, 1947.
5. Lein, A. Endocrinology **32**: 429, 1943.

6. Vanderlaan, W. P. and A. Bissell. Endocrinology **38**: 308, 1946; **39**: 157, 1946.
7. Wolff, J., I. L. Chaikoff, A. Taurog and L. Rubin. Endocrinology **39**: 140, 1946.
8. Vanderlaan, J. E. and W. P. Vanderlaan. Endocrinology **40**: 403, 1947.
9. Salter, W. T., R. E. Cortell and E. A. McKay. J. Pharmacol. and Exper. Therap. **85**: 310, 1945.
10. de Robertis, E. and R. Grasso. Endocrinology **38**: 137, 1946.
11. Miller, W. H., R. O. Robbin, Jr. and E. B. Astwood. J. Am. Chem. Soc. **67**: 2201, 1945.
12. Astwood, E. B. J. Pharmacol. and Exper. Therap. **78**: 79, 1943.
13. Mackenzie, C. G. Endocrinology **40**: 137, 1947.
14. Taurog, A., I. L. Chaikoff and A. L. Franklin. J. Biol. Chem. **161**: 537, 1945.
15. Sadhu, D. P. and S. Brody. This Journal. **151**: 130, 1947.
16. Sadhu, D. P. Univ. Missouri Agr. Exp. Sta. Res. Bull. 408, 1948.
17. Laufer, L. and E. D. Stewart. Science **105**: 327, 1947.

PULSE RATE RESPONSES TO ACCELERATION UNDER REDUCED BAROMETRIC PRESSURE[1]

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Ritter (1) in 1898 demonstrated a marked increase in pulse rate of men who had undergone moderate degrees of centrifugation. This result has been confirmed many times on man and experimental animals (2–6). Jongbloed and Noyons (2) prevented this pulse reflex by denervation of the carotid sinuses of rabbits.

Gellhorn (7) showed that the cardiac reflexes for maintaining normal blood pressure in the human, while standing erect, were abolished during anoxic anoxia. It was suggested (7) that this effect might have been due to hypocapnea produced by the anoxia. Asmussen and Chiodi (8) indicated that anoxic anoxia of short duration resulted in increased cardiac minute volume (8), but the anemic anoxia of carbon monoxide poisoning abolished this compensation.

The purpose of the present investigation was to determine the degree of change which graded acute anoxia would produce on the tachycardia usually observed after positive acceleration.

METHODS

The centrifuge used on this investigation has been described previously (6). The animals were placed in a 120-liter low-pressure chamber which was fixed to the centrifuge rotor 8 feet from the center of rotation. The chamber could be maintained at any desired subatmospheric pressure and was ventilated at the rate of 20 liters per minute (measured at 760 mm. of mercury). Electrocardiographic and electroencephalic recordings were made continuously. Heart rates were measured at 2- to 5-second intervals. All acceleratory exposures in the series were 4 positive g for 10 seconds.

EXPERIMENTAL

The progressive changes in heart rate due to acceleratory exposures, with and without anoxemia, are shown in figure 1. Control monkeys breathed air at sea level; these subjects showed an increase in heart rate of about 30 beats per minute immediately after a 10-second exposure of 4 g acceleration. Administration of

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[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Virginia.

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oxygen (95 per cent O_2 plus 5 per cent CO_2) for 10 minutes at 225 mm. Hg barometric pressure resulted in 20 beats per minute reduction in basal pulse rate. However, these animals demonstrated an increased pulse of 38 beats per minute due to centrifugation, which was a slightly greater increase than the controls showed after acceleration. Ten-minute exposures to 225 mm. Hg without added oxygen almost abolished the tachycardia of centrifugation. The maximum increase of heart rate under these conditions amounted to only five beats per minute. Recovery to initial pulse rate was slow in all cases except during anoxia, when there was little change in heart rate.

TABLE 1. REDUCED BAROMETRIC PRESSURE—ANOXIA

	NO. OF CASES	BEFORE G[1]	HEART RATE AFTER G[1]	INCREASE
Sea level control	23	213	247	34
290 mm. for 15 min.	69	218	232	26
225 mm. for 15 min.	4	210	208	−2
Return to sea level after above	6	210	223	13
Animals breathing pure oxygen				
Sea level	39	183	232	49
285 mm.	44	195	232	37

[1] 4 g, 10 seconds.

TABLE 2. CARBON MONOXIDE ADMINISTERED

CONC. CO IN ATMOSPHERE	NO. OF CASES	HEART RATE BEFORE G[1]	HEART RATE AFTER G[1]	HEART RATE INCREASE
vol. per cent				
0.0%	4	225	250	25
0.16%/50 min.	4	225	245	20
0.3%/15 min.	3	230	240	10
0.3%/40 min.	2	185	192	8
Pure O_2 administration for 7 min. after above	2	190	200	10

[1] 4 g, 10 seconds.

Table 1 demonstrates that change in heart beat after 10 seconds at 4 g was roughly in inverse proportion to the degree of anoxia. Furthermore, recovery after a short period of anoxia is not immediate.

Table 2 shows that 60-minute exposure to 0.16 per cent carbon monoxide under sea-level conditions did not markedly influence the reflex tachycardia of these monkeys during acceleration. Nevertheless, 0.3 per cent carbon monoxide for 15 minutes decreased the usual heart rate response to centrifugation, and 45 minutes exposure to this concentration markedly reduced the pulse rate as well as the acceleration response.

Table 3 demonstrates a synergistic effect of combined anoxic and anemic anoxia with respect to the tachycardia after positive acceleration. These

TABLE 3. HEART RATE WITH ACCELERATION DURING COMBINED ANEMIC AND ANOXIC ANOXIA (TWO MONKEYS)

0.16 vols. per cent CO and 307 mm. barometric pressure

DURATION OF ANOXIA	HEART RATE BEFORE G[1]	HEART RATE AFTER G[1]	HEART RATE INCREASE
min.			
0	210	228	18
0.3	210	232	22
1.0	202	212	10
3.0	200	205	5
7.0	190	183	−7

[1] 4 g, 10 seconds.

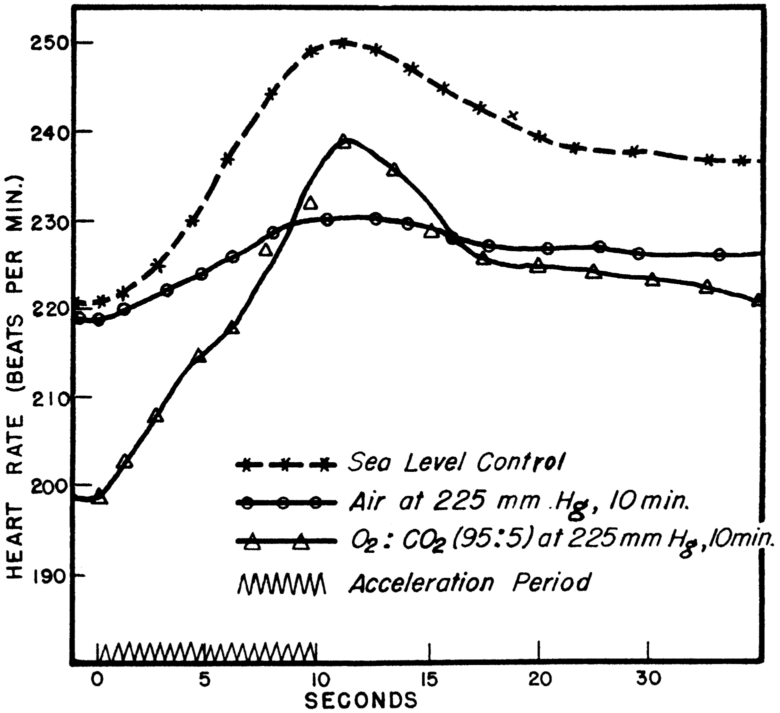

FIG. 1. PULSE RATE DURING AND AFTER ACCELERATION under reduced barometric pressure. Averages of four monkeys exposed to 4 g for 10 seconds.

monkeys were exposed to 307 mm. of mercury barometric pressure (23,000 feet) in addition to 0.16 per cent carbon monoxide (0.5 mm. of carbon monoxide). After two minutes there was a definite retardation of the cardiac reflexes to acceleration. After four minutes there resulted an actual decrease in heart rate following 4 g for 10 seconds.

In the course of these experiments it was observed that rapid recompression (from 282 to 760 mm. Hg in five seconds) resulted in a severe bradycardia in the experimental monkeys. Figure 2 shows that within 30 seconds the pulse rate decreased from 220 per minute to 50 beats per minute. Usually two to three minutes elapsed before the pulse rate was again normal. However, if the animals were subjected to acceleration during the bradycardia period, the usual tachycardia response was immediately demonstrated.

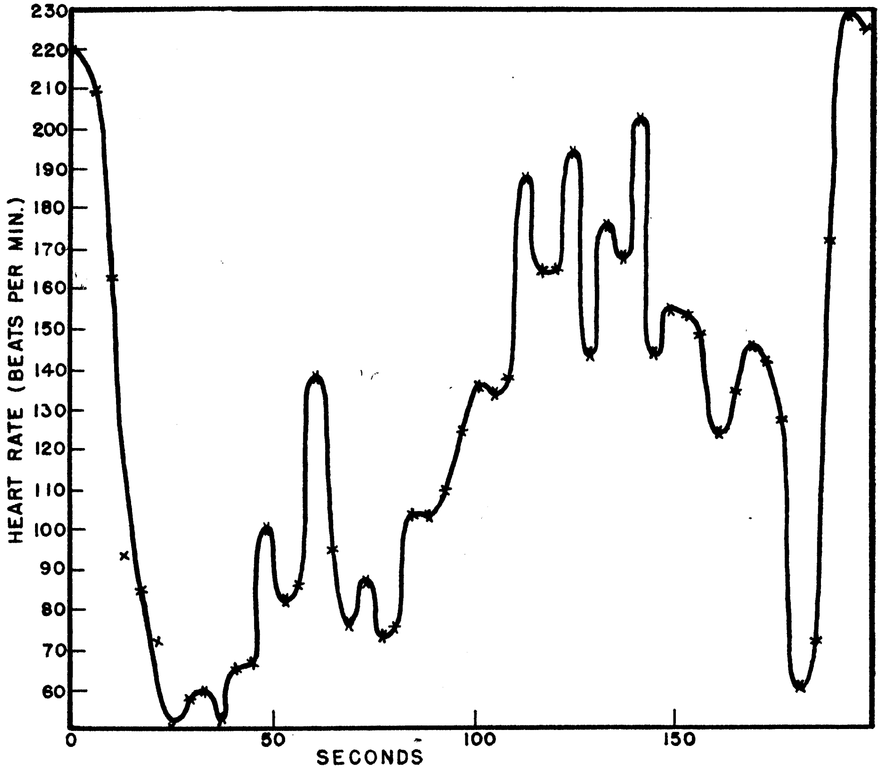

Fig. 2. Erratic pulse after rapid *recompression.* Barometric pressure rose from 282 mm. to 760 mm. Hg within first five seconds. Average results from four monkeys. When animals were centrifuged and recompressed at the same time, this bradycardia did not occur.

DISCUSSION

From these experiments it appears that the tachycardia of positive acceleration may be an objective measure of the integrity of the cardiac reflex system during anoxia, especially if adequate controls are compared. It may be that the tachycardia is dependent solely on blood pressure changes and not indirectly upon anoxia, since the two factors act in opposite directions.

Mild carbon monoxide anoxia may be extremely harmful at moderately high altitudes or in animals suffering from pulmonary embarrassment.

The bradycardia of recompression illustrated here may have been caused by the relatively great and sudden pressure change (478 mm. of mercury) on the pressor receptors, i.e., the carotid sinuses.

CONCLUSION

Administration of oxygen during acceleration at reduced barometric pressure tends to improve the cardiac reflexes. On the other hand anoxia inhibits these reflexes, and the degree of inhibition is directly related to the severity of the oxygen lack. Partial loss of cardiac reflexes may be more than additive when anoxic anoxia is intensified with mild carbon monoxide poisoning.

The author is grateful to Doctors S. W. Britton and V. A. Pertzoff for their assistance, advice and encouragement. R. F. Kline and C. R. French furnished invaluable technical aid throughout the investigation.

REFERENCES

1. Ritter, von Fredrich. Wiener klin. woch. **11:** 361, 1898.
2. Jongbloed, J. and A. K. Noyons. Pfluger's Arch. ges. Physiol. **233:** 67, 1933.
3. von Diringshofen, H. and B. Belonoshkin. Z. Biol. **93:** 79, 1932.
4. von Diringshofen, H. Z. Biol. **95:** 155, 1934.
5. Wood, E. H., E. H. Lambet, E. J. Baldes and C. F. Code. Federation Proc. **5:** 327, 1946.
6. Britton, S. W., V. A. Pertzoff, C. R. French and R. F. Kline. This Journal **150:** 7, 1947.
7. Gellhorn, E. Ann. Internal Med. **10:** 1267, 1932.
8. Asmussen, E. and H. Chiodi. This Journal **132:** 426, 1941.

PHYSIOLOGICAL STUDIES OF THE EFFECTS OF INTERMITTENT POSITIVE PRESSURE BREATHING ON CARDIAC OUTPUT IN MAN[1, 2]

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Three types of positive pressure breathing have been differentiated: *a*) continuous positive pressure breathing where a pressure above atmospheric is maintained in the lungs throughout the respiratory cycle, *b*) expiratory positive pressure breathing in which a pressure above atmospheric is present during the expiratory phase of breathing, and *c*) intermittent positive pressure breathing (IPPB), provided by automatic respirators, which consists in an active inflation of the lungs under an increasing positive (above atmospheric) pressure; while after cycling of the respirator, deflation of the lungs occurs almost to or to atmospheric pressure as a passive process engendered by the elasticity of the lungs and chest-wall structures. Only the latter type of pressure breathing (IPPB) is suitable for maintaining artificial respiration in the apneic subject. Moreover, IPPB as produced by automatic respirators is more comfortable and less exhausting for the conscious subjects when compared to continuous or expiratory positive pressure.

In a number of recent studies measurements of cardiac output were made on the same subjects during normal breathing at ambient pressure and during positive pressure breathing. Several observers (1, 2, 3,) have reported a reduction in cardiac output during positive pressure breathing in man, more or less proportional to the mean mask pressure used. Previous studies from this laboratory (4, 5) have shown that the changes in cardiac output are quite variable depending upon the types of intermittent positive pressure-breathing respirators used. In the same individual the cardiac output was decreased when on one type of respirator and increased when on another type. It has been suggested that these differences are related to the shape of the mask pressure curve produced by the intermittent positive pressure-breathing respirators (5).

In the present study, instead of grouping the data according to the type of automatic respirator used, the changes in cardiac output observed have been

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correlated to the type of mask pressure curve obtained. An attempt is then made to analyze the physiological factors involved in such correlation.

METHODS

The following automatic respirators were used to produce intermittent positive pressure breathing (IPPB): *a*) the pneumatic balance respirator, Burns model, PBR (6, 3), *b*) the Bennett aviation model respirator, Ben. X–1 (4, 5), *c*) the Bennett clinical research model respirator, Ben. X–2 (4, 5) and *d*) the Emerson intermittent positive pressure model (4, 5). All of the above respirators except the Emerson followed the slightest breathing efforts of conscious patients, and subjectively the action was smooth and the harshness of the 'suck and blow' type, with a fixed cycling pattern, was absent.

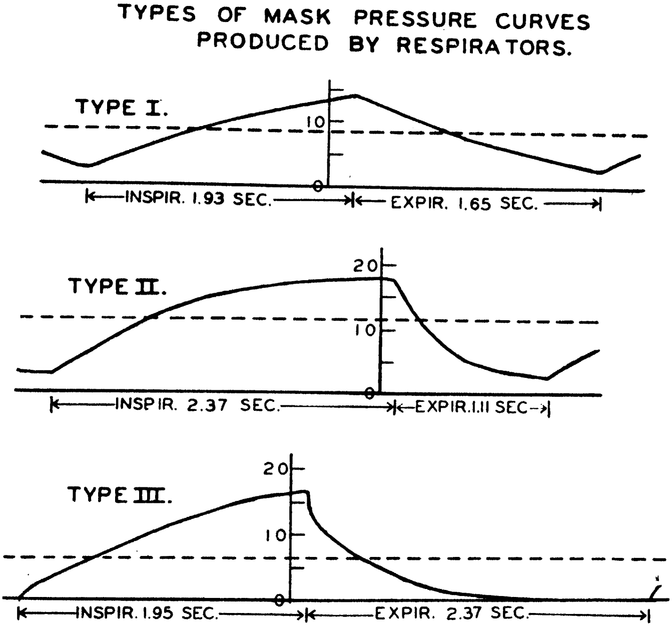


FIG. 1. THREE TYPES OF MASK PRESSURE CURVES observed during IPPB studies. The dotted line is the mean mask pressure for the entire respiratory cycle.

All of the mask pressure curves provided with IPPB have been classified in one of three types as shown in figure 1: Type I-IPPB consists of a symmetrical mask pressure curve with a gradual increasing and decreasing slope, with inspiratory and expiratory time approximately the same, and with a minimal expiratory pressure slightly above atmospheric. Type II–IPPB consists of an asymmetrical mask pressure curve with a rapidly increasing pressure during inspiration and a rapidly decreasing pressure during expiration, with a long inspiratory and a short expiratory time period, and the minimal expiratory pressure above atmospheric. Type III–IPPB consists of an asymmetrical mask pressure curve with a gradually increasing pressure during inspiration and a pressure rapidly dropping to atmospheric early in expiration, and the expiratory time equal to or exceeding inspiratory time. The PBR and the Emerson intermittent positive pressure model produce only type I–IPPB, and the Ben. X–1 produces only type II–IPPB. The Ben. X–2 can be adjusted to produce the three types of mask pressure curves.

Cardiac output was determined by the direct Fick method employing the right heart catheterization technique (7–10) with the subject resting supine in a basal state. Control cardiac output measurements were determined at ambient pressure (normal spontaneous breathing) before and 30 minutes after each period of IPPB. The mixed venous blood was obtained from the right ventricle or pulmonary artery. Measurements were usually obtained after 10 minutes of IPPB; however, in four subjects serial cardiac output measurements were taken over a 70-minute period of continuous IPPB. The arterial blood was obtained through an intra-arterial needle placed in most cases in the brachial and in a few cases in the femoral artery. Sampling of arterial blood and of mixed venous blood and the measurement of oxygen consumption were made simultaneously for each cardiac output determination (10). Oxygen consumption was measured by analysis on the Haldane apparatus of expired air collected in a Tissot spirometer. In oredr to make all the calculations comparable, the inspired air was supplied in all instances from a compressed air cylinder, the gas content of which was checked by repeated analyses.

Simultaneous pressure tracings were recorded from the right heart, peripheral artery and face mask using Hamilton manometers (11) or an electric recording system (12). In addition, intrapleural pressures were also recorded in five cases with a therapeutic pneumothorax. The mean pressures were determined by planimetric integration of the area under the curve for complete respiratory cycles for each of the various tracings recorded.

The Van Slyke-Neill apparatus was used to analyze the blood samples for oxygen content and capacity and carbon dioxide content. The blood pH_s was determined directly using a glass electrode and the pCO_2 was calculated.

RESULTS

The data obtained in 33 IPPB experiments on 29 human subjects with essentially normal circulation are recorded in table 1.

1. *Mask pressure.* The mean mask pressure with type II was almost twice that with type III and 50% greater than with type I, although the average peak mask pressure was only slightly higher. However, for a given peak mask pressure (the significant one in inflating the lung in artificial respiration) the mean mask pressure will be higher for type II than for type III as can be seen from the shape of the respective curves (fig. 1), with type I mask pressure curve being intermediate between the other two types.

2. *Ventilation.* During IPPB both abdominal and thoracic respiratory excursions were increased with a preponderance of diaphragmatic movement as viewed with the fluoroscope. The respiration rate was decreased the most with type I

(33%) and the least with type III (18%, table 1). The minute volume of ventilation was only slightly increased with type I (by 23%), but markedly increased with type II (by 67%), and type III (by 132%). The ventilation rise was due to an increase in tidal volume, since the respiration rate was decreased with all three types. The pH_s increased from 0.05 units with type I to 0.12 units with type II, and accordingly the calculated pCO_2 decreased 4 and 13 mm. Hg, respectively. The correlation was good between the degree of hyperventilation

TABLE 1. EFFECT OF THREE TYPES OF INTERMITTENT POSITIVE PRESSURE BREATHING, AS DIFFERENTIATED BY THE SHAPE OF THE MASK PRESSURE CURVE, ON VENTILATION, BLOOD PRESSURE AND CARDIAC OUTPUT

	TYPE OF MASK PRESSURE CURVE		
	I	II	III
	Number of experiments		
	16	10	7
Peak mask pressure (mm. Hg)	14.2	16.7	12.3
Mean mask pressure (mm. Hg)	7.0	10.6	5.7
Respiration rate			
per min.	14	13	12
per cent change[1]	−33	−28	−18
Ventilation rate			
Liters per min.	10	13	16
per cent change[1]	+23	+67	+132
Arterial blood, pCO_2 and pH_s			
(1) pCO_2			
mm. Hg	34	28	30
change[1] mm. Hg	−4	−13	−8
(2) pH_s			
pH_s	7.47	7.55	7.50
change[1] in pH_s units	+.05	+.12	+.08
Blood pressure, syst/diast. (mm. Hg)			
(1) Arterial			
Normal breathing	129/77	126/77	129/77
Pressure breathing	135/82	123/79	133/82
(2) Right ventricle			
Normal breathing	28/2.5	25/1	28/2
Pressure breathing	29/5	30/8	33/5
Cardiac output			
Liters per min.	5.9	5.8	6.3
per cent change[1]	−14.5	−16.5	+6.0

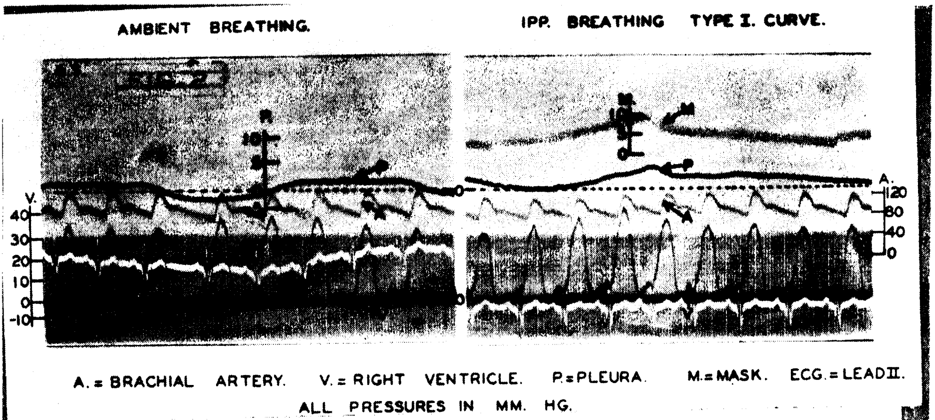
[1] With respect to normal breathing at atmospheric pressure.

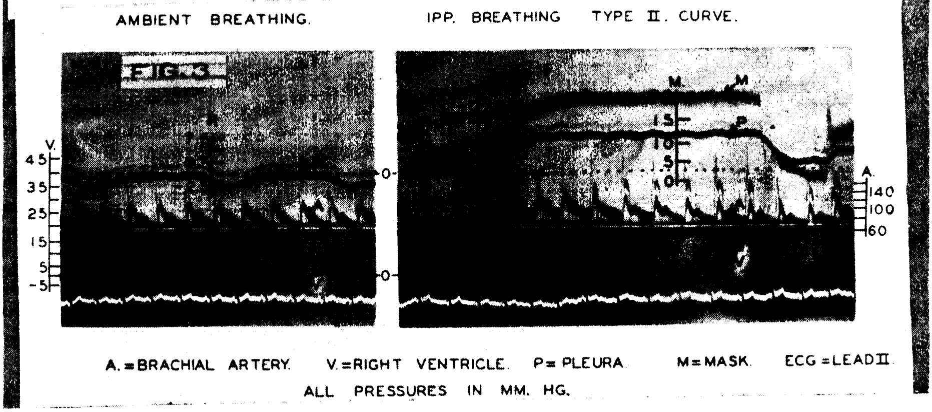
produced by types I and II-IPPB and the change in pCO_2. Although the hyperventilation was most marked with type III–IPPB, there was a larger dead space in the hose-line connection of the mask to the respirator, with a resulting greater rebreathing; this probably accounted for the observed smaller change in pCO_2 and pH_s. Changes in blood gases and pH_s observed after the respirators were in use for several hours were of the same magnitude as the changes observed after 10 to 15 minutes. The respiratory alkalosis observed was apparently of little clinical significance. Even in the cases on type II-IPPB, where the respiratory changes from hyperventilation were most marked (pCO_2 28 mm. Hg and pH_s 7.55), no manifestation of tetany was observed. Observations after 60 minutes (3) on subjects at a 25,000-foot simulated altitude and with hyperventilation showed still lower pCO_2 and a higher pH_s without evidence of tetany.

3. *Blood pressures.* The blood-pressure changes produced by the three types of IPPB were very small as shown in table 1 and figures 2, 3 and 4. There was a slight rise in the right ventricle blood pressure with all three types. The right ventricle end diastolic pressure rise was the largest with type II (7 mm. Hg as compared to 2.5 and 3.0 mm. Hg with the others). The systolic pressure rise was the least with type I. The arterial pressure, both systolic and diastolic, was increased slightly with types I and III and decreased by 3 mm. Hg with type II. The latter decrease may be related to the decrease in pulse pressure present after about five heart beats after inspiration starts, which persists over the balance of the long inspiratory period and is followed by an increase in pulse pressure over only a short expiratory period.

4. *Cardiac output.* The cardiac output was decreased on an average by 14.5 per cent with type I–IPPB and 16.5 per cent with type II-IPPB when compared to control cardiac output measurements made during ambient breathing at atmospheric pressure. There was no decrease with type III-IPPB as compared to the controls but a slight increase of 6.0 per cent. When the decrease in cardiac output, which occurred with types I and II, was plotted against the mean mask pressure for each individual case, there was a good correlation between the height of the mean mask pressure and the decrease in cardiac output (figs. 5 and 6). There was no decrease in cardiac output with type III, hence no correlation between the height of the mean mask pressure and the change in cardiac output as with the other two types of IPPB (fig. 7).

The effect of a 70-minute period of continuous IPPB with type I curve was studied on four essentially normal subjects by serial cardiac output determinations. The cardiac output was taken 10 minutes after starting type I–IPPB, after 40 minutes and after 70 minutes. Thirty minutes after the IPPB was stopped, the final ambient cardiac output measurement was taken. The decrease in cardiac output was greater during the first 10-minute period (23%) as compared to a 17 per cent decrease present after the 40-minute and 70-minute periods of IPPB. Most of the studies of the effects of IPPB on cardiac output have been made after 10 minutes of pressure breathing, which apparently is a period of maximal change in cardiac output for any given type and pressure of IPPB employed.





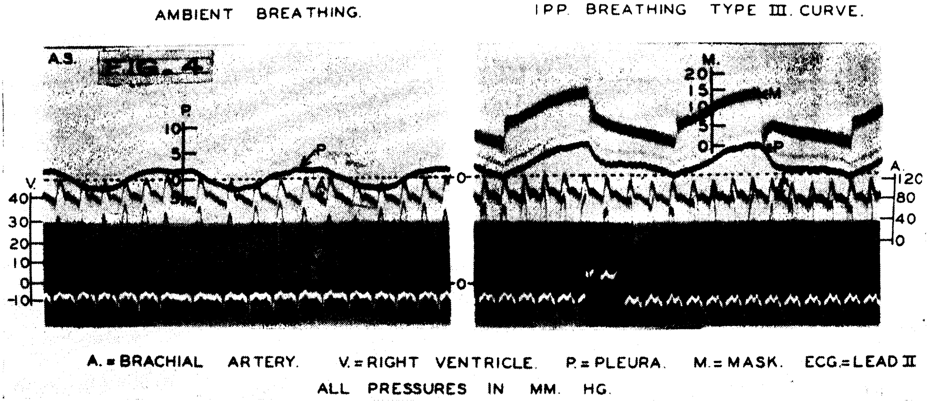
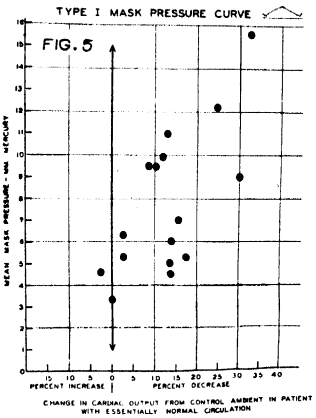


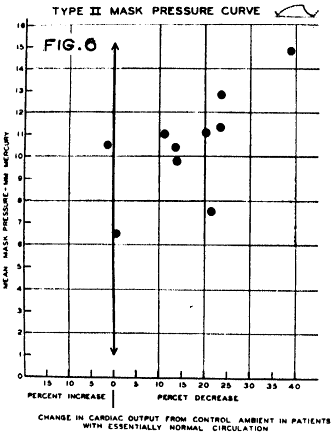
FIG. 2. SIMULTANEOUS PRESSURE RECORDINGS with Hamilton manometers and the electrocardiogram.

FIG. 3. SIMULTANEOUS PRESSURE RECORDINGS with Hamilton manometers and the electrocardiogram. Note the decrease in arterial pulse pressure during the latter half of inspiration with type II-IPPB.

FIG. 4. SIMULTANEOUS PRESSURE RECORDINGS with Hamilton manometers and the electrocardiogram. Note there are as many heart beats during expiration as during inspiration.

5. *Net pressure filling in right ventricle.* In an attempt to analyze the mechanism underlying the changes observed in cardiac output, net filling pressure measurements in the right ventricle were made during IPPB in three subjects with a therapeutic pneumothorax. The net filling pressure for the right ventricle for a given heart beat was calculated by subtracting the simultaneously recorded pleural pressure from the pressure in the right ventricle at the end of diastole. A beat-to-beat analysis was made of mask pressure, pleural pressure, net filling pressure of the right ventricle and the duration of the cardiac cycle for consecutive pulses and for complete respiratory cycles, and representative graphic analyses have been made for both ambient and IPPB as shown in figure 8 for type I, in figure 9 for type II and in figure 10 for type III. It can be seen that the curves of net filling pressure, although in phase, varied inversely as the pleural pressure. The net filling pressure of the right ventricle decreased during the phase of increasing mask pressure and increased during the phase of decreasing mask pressure. It is well shown in figures 9 and 10 that the variations in net filling pressure were not due to lengthening or shortening of the duration of the cardiac cycle.





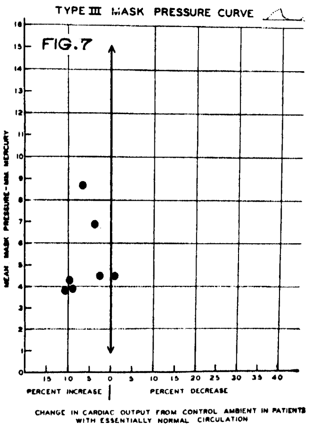
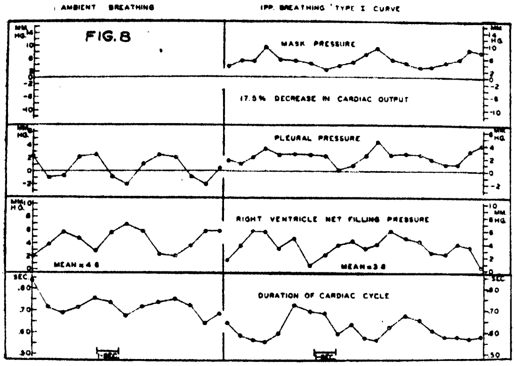


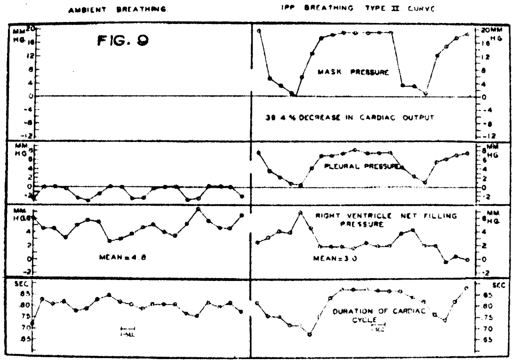
FIG. 5. RELATION BETWEEN MEAN MASK PRESSURE INCREASE and change in cardiac output from control ambient. Note the proportional decrease in cardiac output as the mean mask pressure is increased.

FIG. 6. NOTE A SIMILAR PROPORTIONAL DECREASE in cardiac output as the mean mask pressure is increased as shown in figure 5.

FIG. 7. NOTE THE ABSENCE OF A DECREASE in cardiac output correlated with the mean mask pressure as shown in figures 5 and 6.

It was also found that when the *mean* right ventricular net filling pressure calculated for the several entire respiratory cycles was decreased during IPPB as compared to the control value, there was also a decrease in cardiac output, as observed with type I and II–IPPB (figs. 8 and 9). The greater the decrease in the *mean* right ventricular net filling pressure, the greater the decrease in cardiac output. On the other hand, when the *mean* right ventricular net filling pressure for entire respiratory cycles was unchanged or slightly increased as compared to the control value, there was no decrease in cardiac output as observed with type III-IPPB (fig. 10). All these observations made with two types of IPPB on each of the three subjects with a therapeutic pneumothorax are summarized in figure 11. In this figure the differences between the *mean* right ventricular net filling pressure measured for several respiratory cycles during ambient pressure breathing and IPPB are plotted against the corresponding differences in cardiac output. It shows a very good correlation between changes in the right ventricular net filling pressure and cardiac output. It can be seen that a decrease of 0.5 and 1.8 mm. Hg in the mean right ventricular





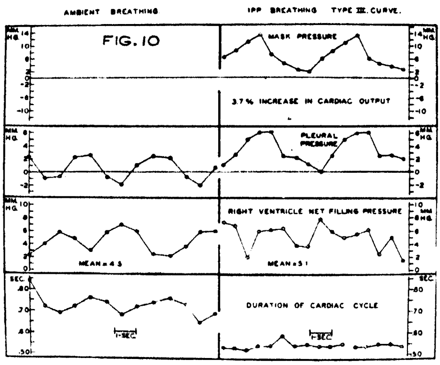


FIG. 8. A BEAT-TO-BEAT ANALYSIS giving the correlation between pleural pressure and hemodynamic changes with ambient and respirator breathing. The mean net-filling pressure was measured over several respiratory cycles both by planimetric integration of the curves and by averaging the summated values of each separate beat. The difference between the mean values calculated by both methods was insignificant. The cardiac output was measured immediately before and after the pressure records were taken.

FIG. 9. A BEAT-TO-BEAT ANALYSIS giving the correlation between pleural pressure and hemodynamic changes with ambient and respirator breathing. For further explanation see figure 8 and text.

FIG. 10. A BEAT-TO-BEAT ANALYSIS giving the correlation between pleural pressure and hemodynamic changes with ambient and respirator breathing. For further explanation see figure 8 and text.

net filling pressure resulted in a reduction in cardiac output of 25 per cent and 39 per cent, respectively, while an increase of 0.5 mm. Hg in the mean right ventricular net filling pressure resulted in an increase of 12 per cent in cardiac output.

Right intraventricular pulse pressure variations were also studied in relation to the shape of the mask pressure curve, but no consistent relationship was found. It should be pointed out that in contrast to net filling pressure, pulse pressure in the right ventricle is influenced both by stroke volume and pulmonary vascular resistance changes. The effects of a changing stroke volume upon the pulse pressure in the right ventricle may be masked by the effects in opposite directions of variation in pulmonary vascular resistance caused by IPPB. Therefore, no conclusion may be derived concerning stroke volume variations during IPPB from a beat-to-beat analysis of pulse pressures in the right ventricle.

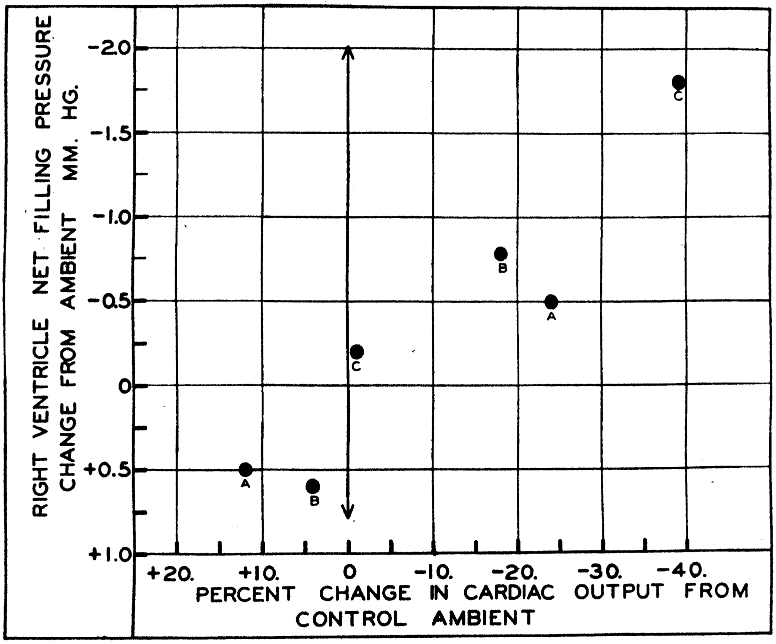


FIG. 11. RELATIONSHIP BETWEEN THE MEAN RIGHT VENTRICULAR NET-FILLING PRESSURE for complete respiratory cycles and the changes produced in cardiac output. The three cases plotted above on the graph are designated by the letters *A*, *B* and *C*. Each letter refers to the same case studied with two types of IPPB.

DISCUSSION

It is well known that variations in intrathoracic pressure may have an influence upon the filling pressure and therefore the output of the right heart. Hamilton *et al.* (13) found that the pressure gradient forcing blood through the lungs is decreased by a prolonged rise in intrathoracic pressure and increased immediately afterwards in the unanesthetized dog. Lauson, Bloomfield, and Cournard (14) have given evidence indicating that the stroke volume of the two ventricles is unequal in man during deep breathing. The following mechanism was suggested: with an increase in pressure in the thorax during expiration, the right heart filling pressure from the large veins was reduced and the stroke volume decreased. At the same time the output from the left heart increased during expiration with the increased venous return from the pulmonary circuit. Studies of IPPB indicate that the same mechanism is operating in a reverse fashion and to a greater extent with the phase of increasing intrathoracic pressure during inspiration and the phase of decreasing pressure during expiration. The decrease in output from the right heart, which occurs during the phase of rising pressure in the mask and in the thorax, may be made up during the phase of falling mask pressure. The reverse occurs in the left heart but with limitations, for the increased output during inspiration with IPPB can be maintained for short periods only (3–5 pulses) by the displacement of pulmonary blood, for if the inspiratory mask pressure is prolonged, the arterial pulse pressure decreases after the initial rise (fig. 3). Thus the variation in stroke volume in the right ventricle is the important part to consider in relation to changes in cardiac output.

The right ventricular net diastolic filling pressures were lower with the IPPB during periods of increasing intrapleural pressure than during the periods of ambient breathing with all three types of mask pressure curves. The reverse occurred during the period of decreasing intrapleural pressure when the mask pressure decreased to or almost to atmospheric. If one may apply here what is commonly referred to as Starling's principle[5] in the sense that increased diastolic filling pressure results in increased stroke volume, then these changes suggest that the deficit in cardiac output incurred during the phase of increased intrapleural pressure is compensated for during the phase of decreased intrapleural pressure. If after cycling of the respirator the pressure falls rapidly to atmospheric and if the duration of expiration is slightly longer than inspiration, then compensation may be complete and no decrease in cardiac output occurs, as observed with type III–IPPB. Compensation during expiration is not, however, complete with types I and II-IPPB, because the pressure does not drop rapidly to atmospheric with type I-IPPB and expiratory time is too short, and the end pressure is still above atmospheric with type II-IPPB.

An overall confirmation of Starling's principle is provided by the observations on *mean* net filling pressure in the right ventricle measured over entire respiratory cycles. Accordingly, in these observations a change in this *mean* net filling pressure is associated with a proportional change in cardiac output and it is well to emphasize that even small changes, demonstrated with accurate methods of recording pressure in the heart, may have a considerable effect upon cardiac output.

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[5] Actually this pressure-flow relationship was proposed by O. Franck in 1904, and discussed by Patterson, Piper and Starling in 1914, who put forth the proposition that the fiber length or diastolic volume rather than the diastolic filling pressure determined the energy of mechanical ejection.

The question may be raised as to whether the intrapleural pressure values are the same as the extracardiac pressures during both phases of respiration or vary in the same direction. Bookhart and Boyd (15) have reported higher pressures inside the pericardium than in a small closed pneumothorax in anesthetized dogs, but on enlarging the pneumothorax the pressure difference was decreased. It would appear that the pressure in a therapeutic pneumothorax in man involving one fourth to one third of the volume of one lung would approximate very closely the pressure outside the right auricle.

On the basis of the observations made with the three types of IPPB on changes in cardiac output in essentially normal unanesthetized subjects, a very desirable type of IPPB mask pressure curve produced by respirators may be described. The rise in pressure should gradually increase during inspiration to a peak not in excess of approximately 25 cm. water, and after cycling occurs, the pressure drop should be rapid early in expiration and with a mean mask pressure during the expiratory period as near atmospheric as possible, as with type III-IPPB (fig. 1). Inspiratory time should not exceed expiratory time, thereby allowing as many heart beats to occur during expiration, with the increased right ventricular net filling pressure, as during inspiration, with the decreased net filling pressure. Such a mask pressure curve as described above permits the decrease in output from the right heart during the inspiratory phase of rising intrathoracic pressure to be made up during the phase of falling mask pressure.

SUMMARY

1. The effects of three types of intermittent positive pressure breathing, as differentiated by the shape of the mask pressure curve, have been correlated with the changes in cardiac output observed in 33 experiments on 29 human subjects.

2. The three types of mask pressure curves were as follows: type I, symmetrical with gradual increasing and decreasing slope, expiratory time approximately the same as inspiratory and the end expiratory pressure above atmospheric; type II, asymmetrical with rapidly increasing pressure during inspiration and rapidly dropping during expiration, long inspiratory and short expiratory time intervals and the end expiratory pressure above atmospheric; and type III, asymmetrical with gradually increasing pressure during inspiration and suddenly dropping early in expiration to atmospheric and expiratory time equal to or exceeding inspiratory.

3. Cardiac output was decreased more or less in proportion to the increase in mean mask pressure with the first and second type curves. There was no decrease in cardiac output with the third type curve.

4. The net filling pressure of the right ventricle decreased during the phase of increasing mask pressure and increased during the phase of decreasing mask pressure with all types of curves. The curve of net filling pressure of the right

ventricle, although in phase with, varied inversely as the pleural pressure and mask pressure.

5. The mean net filling pressure of the right ventricle was calculated for complete respiratory cycles during intermittent pressure breathing and during ambient breathing. In cases where the cardiac output was reduced during intermittent pressure breathing as compared to ambient, the mean net filling pressure was reduced also. Conversely, when the cardiac output was increased the mean net filling pressure was increased.

6. Interpreted in terms of variation in stroke volume, these changes suggest that the deficit in cardiac output incurred during the inspiratory phase is compensated for during the expiratory phase. When the pressure drop is rapid in expiration with the resulting intrapleural pressure low and the right ventricular net filling pressure high, compensation is complete provided expiratory time is of sufficient duration. Expiratory time must equal or exceed inspiratory time in order that the number of heart beats during expiration may equal or exceed the number present during inspiration. The time and pressure relationships with the third type of curve permits compensation to be complete, as the mean right ventricular net filling pressure is not decreased. Compensation was incomplete with the first and second types, because the mask pressure does not drop rapidly after cycling with the first, expiratory time is too short and the end expiratory pressure is still above atmospheric with the second.

7. A very desirable type of IPPB should provide a mask pressure curve that shows: *a*) a gradual increase in pressure during inspiration, *b*) a rapid drop in pressure after cycling occurs, *c*) a mean mask pressure during the expiratory period as near atmospheric as possible and *d*) an expiratory time equal to or exceeding the inspiratory time. Adequate ventilation can be provided with the above type of pressure breathing in man with a minimal disturbance to the circulation, and this type would seem most desirable physiologically for administering artificial respiration.

REFERENCES

1. Barach, A. L., M. Eckman, E. Ginsburg, C. C. Rumsey, Jr., I. Korr, I. Eckman and G. Besson. J. Aviation **17**: 290, 1946.
2. Otis, A. B., H. Rahn, M. Brontman, L. J. Mullins and O. W. Fenn. J. Clin. Invest. **25**: 413, 1946.
3. Motley, H. L., A. Cournand, M. Eckman and D. W. Richards, Jr. J. Aviation Med. **17**: 431, 1946.
4. Motley, H. L., A. Cournand, L. Werko, A. Himmelstein, D. Dresdale, R. Johnson, M. Lester, B. Spierto and D. W. Richards, Jr. Memorandum Report, TSEAA-696-79-F, AAF, Hdgs. A.M.C. Eng. Div.
5. Motley, H. L., A. Cournand, L. Werko, D. Dresdale, A. Himmelstein and D. W. Richards, Jr. J. Am. Med. Assoc. In press, 1948.
6. Burns, H. L. Air Surg. Bull. **2**: 306, 1945.
7. Cournand, A. and H. A. Ranges. Proc. Soc. Exp. Biol. and Med. **46**: 462, 1941.
8. Cournand, A., H. D. Lauson, R. A. Bloomfield, E. S. Breed and E. deF. Baldwin. Proc. Soc. Exp. Biol. and Med. **55**: 34, 1944.

9. Cournand, A., R. L. Riley, E. S. Breed, E. deF. Baldwin and D. W. Richards, Jr. J. Clin. Invest. **24**: 106, 1945.
10. Cournand, A. Federation Proc. **4**: 207, 1945.
11. Bloomfield, R. A., H. D. Lauson, A. Cournand, E. S. Breed and D. W. Richards, Jr. J. Clin. Invest. **25**: 639, 1946.
12. Motley, H. L., A. Cournand, L. Werko, D. Dresdale, A. Himmelstein and D. W. Richards, Jr. Proc. Soc. Exp. Biol. and Med. **64**: 241, 1947.
13. Hamilton, W. F., R. A. Woodbury and E. Vogt. This Journal **125**: 130, 1939.
14. Lauson, H. D., R. A. Bloomfield and A. Cournand. Am. J. Med. **1**: 315, 1946.
15. Bookhart, J. M. and T. E. Boyd. This Journal **148**: 434, 1947.

EFFECT OF XANTHOPTERIN ON CELL PROLIFERATION IN BONE MARROW CULTURES

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A comprehensive review of dietary factors concerned in erythropoiesis has recently appeared (1). Of the factors influencing hemapoiesis the pterins and pteroyl derivatives have received considerable attention. In 1936 Tschesche and Wolf (2) reported that xanthopterin would cure the anemia produced in rats by a diet of goat's milk. Norris and Simmons (3) in 1941 found that xanthopterin injected into anemic fish produced a rapid rise in red blood cells to a normal value. Totter and Day (4) in 1943 reported that xanthopterin promotes growth and alleviates the leucopenia in rats fed a purified diet containing 1 per cent succinylsulfathiazole. Other investigators (5–7) claim that they have not been able to confirm the work of Totter and Day.

Hays (8) in 1946 used a modification of Osgood and Brownlee's (9) bone-marrow culture technique to test the effectiveness of pteroylglutamic acid (folic acid, PGA), its heptaglutamyl conjugate and liver fractions in the production of reticulocytes in vitro. They found that PGA or its conjugate did not give a higher reticulocyte value than Tyrode's solution alone, while normal blood serum and liver extracts did. An appreciable quantity of PGA has not been demonstrated in highly potent antipernicious anemia liver fractions (10, 11). In 1940 Norris and Simmons (12) in this laboratory isolated a substance from antipernicious anemia liver preparations following the procedure of Koschara and Haug (13) for the isolation of xanthopterin and tested the preparation on anemic fish. The preparation had an activity equal to synthetic xanthopterin or that isolated from human urine and was also identical in fluorescence.

The present paper is a report on the effect of xanthopterin on cell proliferation in bone marrow cultures.

Bone marrow was obtained from various animals as reported below under as near aseptic conditions as were practical for the work. All equipment used was sterilized. The bone marrow was prepared as a suspension in Tyrode's solution without glucose or with other diluting solutions as indicated below. The suspension was centrifuged to remove coarse material and lipids. This gave a suspension of cells free from debris or emulsion when examined under the microscope. Such a suspension is referred to below as the bone marrow suspension. Two ml. of the uniform bone marrow suspension was pipetted into each of a series of 5 ml. vaccine vials. A solution containing the supplement to be used was then added to each vial and the volume adjusted with Tyrode's solution such that the final volume in each vial in a given series was the same. The volume of solutions added as supplement was usually 0.2 ml. In the first experiments the vials were incubated in an oven at 37°C. and shaken every half hour.

But as the cells sometimes formed clumps which were difficult to break up, in all subsequent experiments the vials were placed upon a rack devised in a Warburg bath and rocked slowly at 37°C. A sterile glass bead was placed in each vial to facilitate stirring and prevent clumping of the cells. In a typical experiment the marrow was aspirated from the sternum and ribs of three rabbits and added to 20 ml. of Tyrode's solution without glucose. The suspension was diluted to approximately 55 ml. with the same Tyrode's solution. After centrifuging, 2 ml. of the cell suspension was placed in each of 25 vials. The vials were divided into groups and to one group 0.2 ml. of Tyrode's solution was added to serve as control. To another group 0.1 ml. of normal blood serum and 0.1 ml. of Tyrode's solution were added. Two-tenths ml. of xanthopterin or of folic acid solution of such concentration as to give 5γ per ml. final concentration in the suspension was added to each of two groups of vials. Cell counts were made at the beginning of the experiment, after 3 hours incubation at 37°C. and after six hours incubation on samples withdrawn with sterile needles. Table 1 gives the average values as increase in red blood cells (RBC) and white blood cells (WBC) per cmm. of suspension, and the reticulocyte value as reticulocytes per 1000 RBC. The values reported here as white blood cells represent the total nucleated cell count by the technic of leucocyte counting and would include the nucleated precursors of the erythrocytes. To illustrate the calculation, the initial average RBC per cmm. for the group supplemented with 5γ per ml. of xanthopterin was 21 × 10^4 with a range of from 19 to 23 × 10^4. After six hours incubation the average was 32 × 10^4, with a range of from 29 to 36 × 10^4, which gives an average increase of 11.0 × 10^4 ± 1.5 × 10^4.

TABLE 1. CELL PROLIFERATION IN RABBIT BONE MARROW CULTURE, AS INFLUENCED BY FOLIC ACID, XANTHOPTERIN AND NORMAL BLOOD SERUM

SUPPLEMENT	3-HOUR INCUBATION			6-HOUR INCUBATION		
	Increase in RBC per cmm.	Increase in WBC per cmm.	Reticulocytes per 1000 RBC	Increase in RBC per cmm.	Increase in WBC per cmm.	Reticulocytes per 1000 RBC
None	—	320	4	20,000	600	7
Folic acid, 5 γ per ml	—	320	4	30,000	560	7
Xanthopterin, 5 γ per ml	50,000	1400	7	110,000	2440	15
Normal blood serum, 0.1 ml	70,000	2320	10	160,000	6640	19

The average initial reticulocyte value on the suspension was 2 per 1000 RBC. The reticulocyte values at the end of six hours incubation period check very well with the values reported by Hays (8) for no supplement, folic acid and normal blood serum. Xanthopterin at 5γ per ml. concentration gives a reticulocyte value similar to that given by normal blood serum.

In the above experiment the initial reticulocyte value was 2 per 1000 RBC, and as there were 210,000 RBC per cmm., there would be about 400 reticulocytes per cmm. After incubation with normal serum for six hours the reticulocyte value was 19 per 1000 RBC, and as the RBC had increased to 370,000 RBC per cmm., there would be about 7000 reticulocytes per cmm. That would give an increase of approximately 6600 reticulocytes per cmm. while the RBC increase was 160,000 per cmm. If each red cell must pass through a reticulocyte stage before maturing, the number of reticulocytes formed and then either matured or remaining must equal the RBC formed. At the time of counting approximately 4 per cent of the RBC produced during the incubation remained in the reticulocyte stage and 96 per cent had already matured. This does not take into account the RBC which might have been destroyed or disappeared

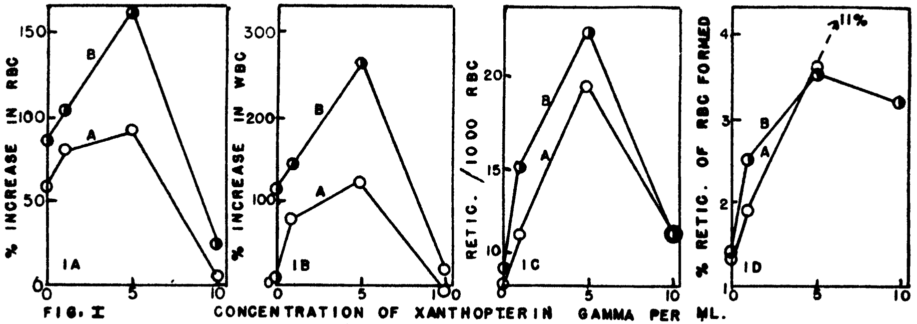

FIG. I

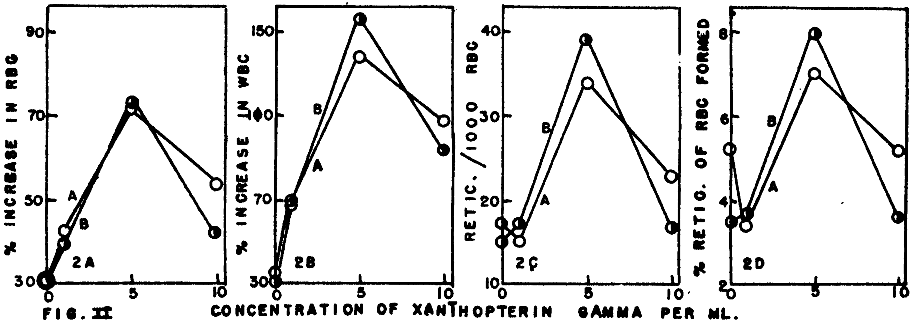

FIG. II

FIG. 1. RESPONSE IN CELL PROLIFERATION of cat bone marrow suspension to added xanthopterin. *Curve A*, seven hours' incubation; *Curve B*, 16 hours' incubation.

FIG. 2. RESPONSE IN CELL PROLIFERATION of beef bone marrow suspension to added xanthopterin. *Curve A*, using Tyrode's solution for dilution; *Curve B*, using Osgood and Brownlee solution for dilution. The time of incubation was five hours.

during the incubation. Similar calculations made with a large number of experiments using rabbit, beef, sheep, rat and cat bone marrow and incubation time ranging from 3 to 16 hours gave an average increase in reticulocytes compared to the total increase in RBC during incubation of about 4 per cent with a range from 1.3 to 11.0 per cent. Over 90 per cent of the RBC formed during incubation had matured at the time of counting. The increased reticulocyte value after incubation of bone marrow in vitro is indicative of rapid cell proliferation but not otherwise significant. The interpretation placed upon the reticulocyte value by Hays (8) is not valid; it cannot be looked upon as only the transformation of 'primitive RBC' originally present in the bone marrow suspension to reticulocytes and the factor or factors influencing it should not be spoken of as a 'primitive RBC maturing factor.'

A concentration of 5γ per ml. of xanthopterin produces a more rapid rate of increase in white blood cells than of erythrocytes, as is also shown in the following experiments.

The effect of xanthopterin upon the cell proliferation in bone marrow cultures is strongly influenced by the final concentration of pterin present. Figures 1 and 2 show the increase in erythrocytes, white blood cells and reticulocytes with varying concentrations of xanthopterin. A maximum rate of proliferation has been noted at a xanthopterin concentration of approximately 5 γ per ml. using various types of bone marrow. There is a rapid decrease in the rate of proliferation with concentrations of xanthopterin above 5 γ per ml. A maximum at approximately 5 γ per ml. has been observed with beef, rabbit, sheep, rat and cat bone marrow. Figure 2 also shows a comparison of the rate of proliferation of bone marrow culture using Tyrode's solution without glucose and using the solution suggested by Osgood and Brownlee (9) without glucose for bone marrow culture. No significant difference was found between the two solutions.

SUMMARY

1. Xanthopterin causes an increase in the rate of cell proliferation of both erythrocytes and total nucleated cells, counted and reported as total white blood cells in vitro, in a bone marrow suspension.

2. The optimum concentration of xanthopterin for proliferation is approximately 5 γ per ml. of bone marrow suspension for beef, rabbit, rat, sheep and cat bone marrow.

3. Xanthopterin causes an increase in reticulocytes in bone marrow culture; the increase in reticulocytes is indicative of rapid erythrocyte proliferation.

REFERENCES

1. Cartwright, G. E. J. Hematology **2:** 111, 1947.
2. Tschesche, R. and H. J. Wolf. Z. Physiol. Chem. **244:** 1, 1936; **248:** 34, 1937.
3. Norris, E. R. and R. W. Simmons. J. Biol. Chem. **140:** 679, 1941; **158:** 449, 1945.
4. Totter, J. R. and P. L. Day. J. Biol. Chem. **147:** 257, 1943.
5. Wright, L. D. and A. D. Welch. Science **98:** 179, 1943.
6. Axelrod, A. E., P. Gross, M. D. Bosse and K. L. Swingle. J. Biol. Chem. **148:** 721, 1943.
7. Daft, F. S. and W. H. Sebrell. Pub. Health Rep., **58:** 1542, 1943.
8. Hays, E. E. Proc. Soc. Exp. Biol. and Med., **63:** 558, 1946.
9. Osgood, E. E. and I. E. Brownlee. J. Am. Med. Assoc., **107:** 123, 1936.
10. Clark, G. W. Am. J. Med. Sci. **202:** 520, 1945.
11. Stokstad, E. L. R. and T. H. Jukes. Proc. Soc. Exp. Biol. and Med. **62:** 112, 1936.
12. Norris, E. R., and R. W. Simmons. Unpublished data.
13. Koschara, W. and H. Haug. Z. Physiol. Chem. **259:** 97, 1939.

XANTHOPTERIN IN RAT ANEMIA PRODUCED BY SULFATHIAZOLE

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A review by Gates (1) has recently appeared giving the chemical and physical properties and physiological aspects of the pteridines. Xanthopterin has been associated with physiological function by a number of authors. In 1936 Tschesche and Wolf (2) reported that xanthopterin would cure the anemia produced in rats by a diet of goat's milk. In 1939 Koschara and Hrubesch (3) and Koschara and Haug (4) investigated the occurrence and distribution of uropterin (xanthopterin) in urine and tissues under normal and pathological conditions, and suggested that it had a function in metabolism. Norris and Simmons (5) in 1941 found that xanthopterin injected into anemic young fish produced a rapid rise in red blood cells to a normal value. Totter and Day (6) in 1943 reported that xanthopterin promotes growth and alleviates the leucopenia in rats fed a purified diet containing 1 per cent succinylsulfathiazole. Other investigators (7–9) claim that they have not been able to confirm the work of Totter and Day.

The present paper is a report of the effect of xanthopterin on the blood picture of rats made anemic by a purified diet plus one per cent sulfathiazole.

Young rats, when weaned, were placed upon a diet consisting of: glucose 72 per cent, casein 18 per cent, cottonseed oil 3 per cent, cod liver oil 2 per cent, salt mixture 4 per cent[1] and sulfathiazole 1 per cent. The following vitamins were added per kilogram of diet: thiamine HCl 10 mgm., riboflavin 20 mgm., pyridoxine 10 mgm., ca-pantothenate 40 mgm., niacin 20 mgm., 2-methyl 1-4 naphthoquinone 4 mgm., biotin 0.01 mgm. and choline-Cl 2 grams.

After 22 days on the diet, four rats had an average red blood cell (RBC) count of 3.4×10^6 per cmm., with observed values of 3.15, 3.32, 3.43 and 3.70×10^6 per cmm., hemoglobin of 6.8 grams per 100 ml. of blood, with observed values of 6.3, 6.5, 7.1 and 7.2 grams per 100 ml. and blood cell volume (hematocrit) of 20 per cent, with observed values of 15.7, 20.9, 20.9 and 23.4 per cent. They were injected with 100 γ of xanthopterin per day for five days. Figure 1 shows the average response of the four animals following the injection.

Another series of rats made anemic on the diet described above and having an average RBC count of 4.3×10^6, hemoglobin of 8.6 grams per 100 ml. and blood cell volume of 22.5 per cent, were divided into groups of six rats each and given a single injection of graded amounts of synthetic xanthopterin. One group was given an injection of 100 γ of synthetic pteroylglutamic acid (PGA). Figure 2 shows the response obtained with 25, 50 and 100 γ of synthetic xanthopterin

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[1] The salt mixture used was the Osborne and Mendel salt mixture (J. Biol. Chem. **37**: 572, 1919) supplemented with 0.161 grams of $CuSO_4 \cdot 5\ H_2O$ for each 134.8 grams of $CaCO_3$ used.

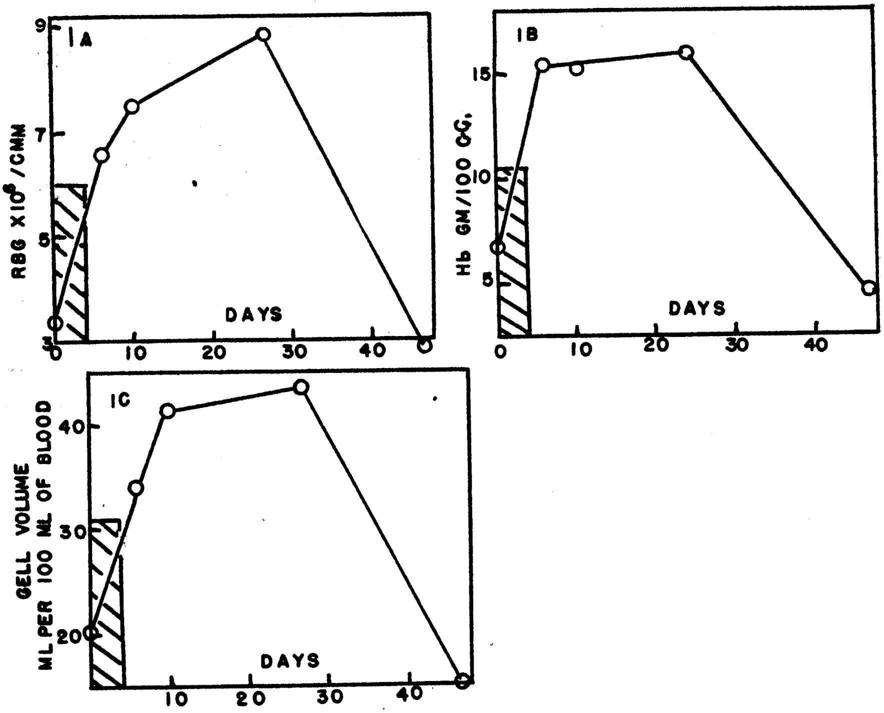

FIG. 1. THE RESPONSE OF YOUNG RATS made anemic on a purified diet containing one per cent sulfathiazole to injection of 100 γ of xanthopterin per day for five days. The shaded portion indicates the period of injection.

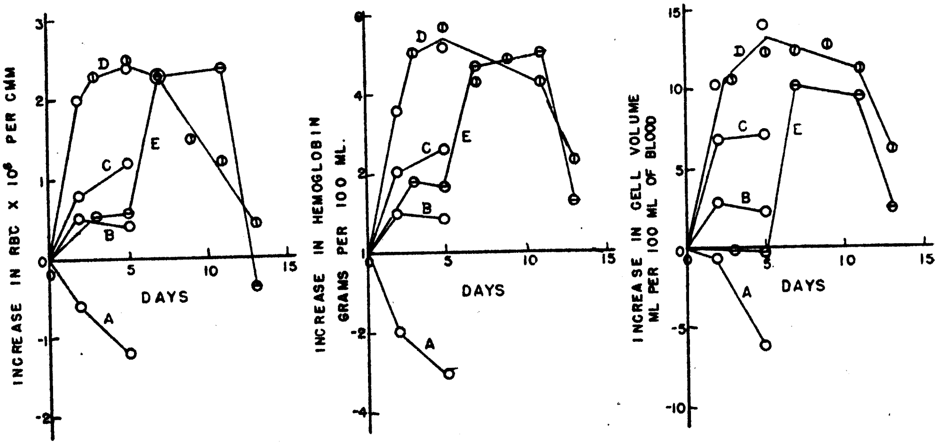

FIG. 2. THE RESPONSE OF YOUNG RATS made anemic on a purified diet containing one per cent sulfathiazole to a single injection of graded amounts of xanthopterin and to pteroylglutamic acid (PGA). *Curve A*, controls with no injection; *Curve B*, 25 γ of xanthopterin; *Curve C*, 50 γ of xanthopterin; *Curve D*, 100 γ of xanthopterin. Open circles are from data obtained using xanthopterin synthesized in this laboratory, circles with vertical cross bar from data obtained with xanthopterin obtained from Dr. J. J. Pfiffner; *Curve E*, circles with horizontal cross bar, 100 γ PGA.

and of 100 γ of PGA. At the 100 γ level of xanthopterin two preparations were used, one synthesized in our laboratory and the other a highly purified preparation obtained from Dr. J. J. Pfiffner, which was used for comparison. At the time of injection the RBC count and hemoglobin were decreasing rapidly. All of the animals of the control group, which had not been injected, died about seven days after starting the experiment. There was a graded response with the three levels of xanthopterin. With xanthopterin there was an immediate rise in RBC, white blood cells (WBC) and cell volume. With the injection of 100 γ of PGA, there was a lag of a few days before a rapid change in the blood picture occurred.

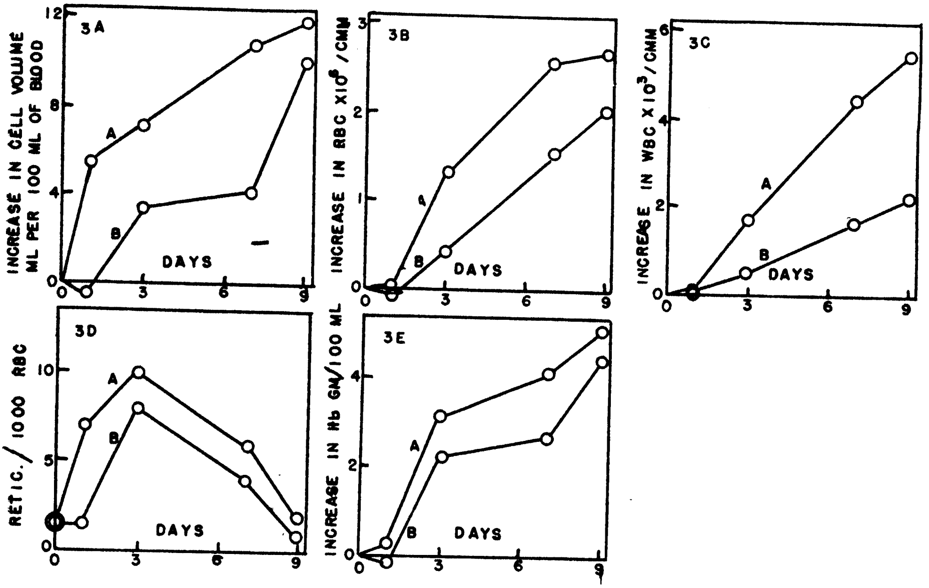

FIG. 3. RESPONSE OF YOUNG RATS made anemic on a purified diet containing one per cent sulfathiazole to a single injection of xanthopterin and pteroylglutamic acid (PGA). *Curve A*, 100 γ xanthopterin; *Curve B*, 300 γ PGA.

Another group of rats made anemic on the diet described above was divided into groups of four rats each and given single injections of 100 γ of xanthopterin and 300 γ of PGA. Three hundred micrograms of PGA was used in the experiment to give a pteroyl concentration of the same order of magnitude as that of the xanthopterin used. Figure 3 shows the results obtained. In this experiment there is also the lag in the response with PGA as compared with the response with xanthopterin, which is especially noticeable in the reticulocyte value and cell volume.

A lag in the response to administration of PGA has been reported in the literature as shown in the recent review of clinical studies by Spies (10) and other reports. A lag in the response of PGA as compared to xanthopterin would also be expected from the work of Norris and Majnarich (11) on bone marrow culture studies. In those experiments xanthopterin was shown to give an immediate and rapid cell proliferation, while PGA did not stimulate cell proliferation. PGA is not used as such by the bone marrow in hemapoiesis while xanthopterin can be utilized. It would appear that before PGA can be utilized by the bone marrow, the pteroyl radicle must first be separated from the conjugated molecule. The pterin is then either used directly or converted by the cells of the marrow into a form or compound which is required in hemapoiesis.

SUMMARY

1. A single dose of xanthopterin injected into rats made anemic on a purified diet containing sulfathiazole produces an immediate and rapid rise in reticulocytes, red blood cells, white blood cells, blood cell volume and hemoglobin.

2. The response to a single injection of xanthopterin is shown to be proportional to the dosage of xanthopterin over the range of dosage used.

3. The lag in response when pteroylglutamic acid (PGA) is injected as a single dose is due to the fact that bone marrow cannot use PGA directly in hemapoiesis while xanthopterin can be utilized.

REFERENCES

1. Gates, M. Chem. Rev. **41:** 63, 1947.
2. Tschesche, R. and H. J. Wolf. Z. Physiol. Chem. **244:** 1, 1936; **248:** 34, 1937.
3. Koschara, W. and A. Hrubesch. Z. Physiol. Chem. **258:** 39, 1939.
4. Koschara, W. and H. Haug. Z. Physiol. Chem. **259:** 97, 1939.
5. Norris, E. R. and R. W. Simmons. J. Biol. Chem. **140:** 679, 1941; **158:** 449, 1945.
6. Totter, J. R. and P. L. Day. J. Biol. Chem. **147:** 257, 1943.
7. Wright, L. D. and A. D. Welch. Science **98:** 179, 1943.
8. Axelrod, A. E., P. Gross, M. D. Bosse and K. L. Livingle. J. Biol. Chem. **148:** 721 1943.
9. Daft, F. S. and W. H. Sebrell. Pub. Health Rep. **58:** 1542, 1943.
01. Spies, T. D. Annual Rev. Biochem. **16:** 387, 1947.
11. Norris, E. R. and J. J. Majnarich. In press.

MAINTENANCE OF VASODILATATION OF THE EXTREMITIES OF NORMAL INDIVIDUALS FOR A PROLONGED PERIOD BY THE INGESTION OF TWO TO FOUR SUBSTANTIAL MEALS IN CLOSE SUCCESSION[1]

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Almost simultaneously, and working independently, Maddock and Coller (1), Herrick and Sheard (2) and Burton and Murlin (3) reported a significant rise in the skin temperature of the fingers and toes following the ingestion of a substantial meal. This rise took place within a half to one hour after the ingestion of food and was maintained generally for two hours or more. Since this effect could be produced by the ingestion of one substantial meal, the question arose as to whether the rise in skin temperature of the fingers and toes could be maintained for a much longer period by the ingestion of three or four substantial meals in close succession.

PROCEDURE

The studies were made on 24 young, normal persons, 3 women and 21 men, whose ages ranged from 18 to 24 years and whose basal metabolic rates ranged from −28 to +7 per cent. Before the tests, the subjects fasted for 15 hours and during the tests they wore lightweight, short pajamas and lay supine on a comfortable bed in a constant temperature room at 25.5°C. (78°F.) with a relative humidity of 40 per cent. The basal metabolic rates were determined before measurements of the skin temperature, and in a few instances the metabolic rate was determined after the ingestion of several meals.

The temperature (4) of the plantar surface of the first and third toes of both feet and the volar side of the distal phalanges of the first and third fingers of both hands was determined by means of copper constantin thermocouples. The observations were made at intervals of ten minutes for seven to eight hours.

Generally, after a control period of approximately one hour, the temperature of the fingers and toes was fairly constant and the first meal was given. The food was usually cooled to a temperature of 37°C. and generally was ingested in 10 to 15 minutes. The meal was balanced in essential types of food and each meal furnished from 500 to 800 calories. Frequently the appetite decreased progressively so that less food was taken at the last meal. After the ingestion of the first meal, from 80 to 120 minutes elapsed before the next meal was given. This interval depended somewhat on the amount of vasodilatation as evidenced

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[1] Read at the meeting of the American Physiological Society, Chicago, Illinois, May 18–22, 1947.

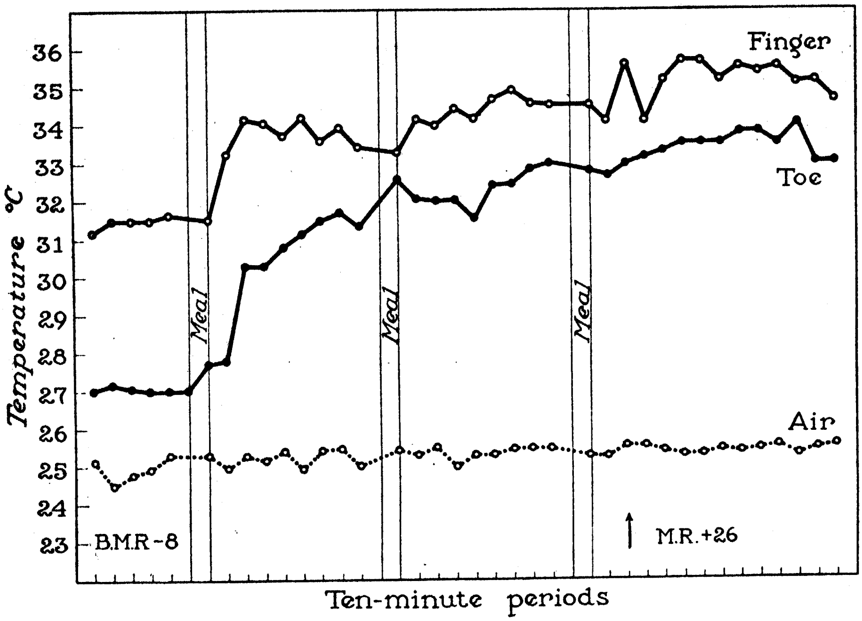

FIG. 1. EFFECT OF THE INGESTION OF THREE MEALS on the skin temperature of the fingers and toes of a normal subject. The skin temperature curves of only one toe and finger are shown. The feet and hands remained dry during the period of vasodilatation. The increased rate of metabolism should be noted.

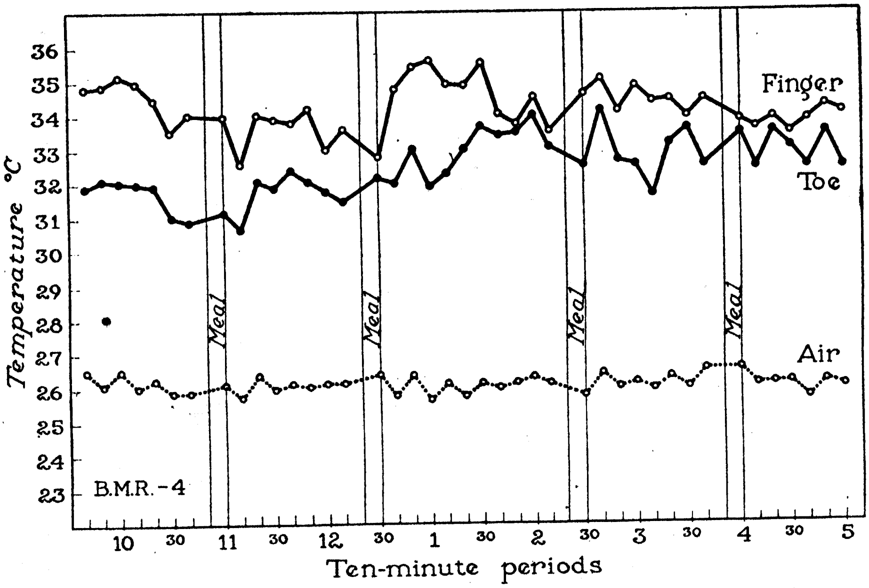

FIG. 2. EFFECT OF THE INGESTION OF FOUR MEALS on the skin temperature of the extremities of a normal subject with a higher basal metabolic rate than the subject whose curve is shown in figure 1. The rise after the ingestion of the food was limited. Some slight sweating on the extremities was present.

by the increase in the skin temperatures of the fingers and toes. Vasodilatation was considered present when the skin temperature of the toes approximated that of the fingers. There is always some gradient from the fingers to the toes.

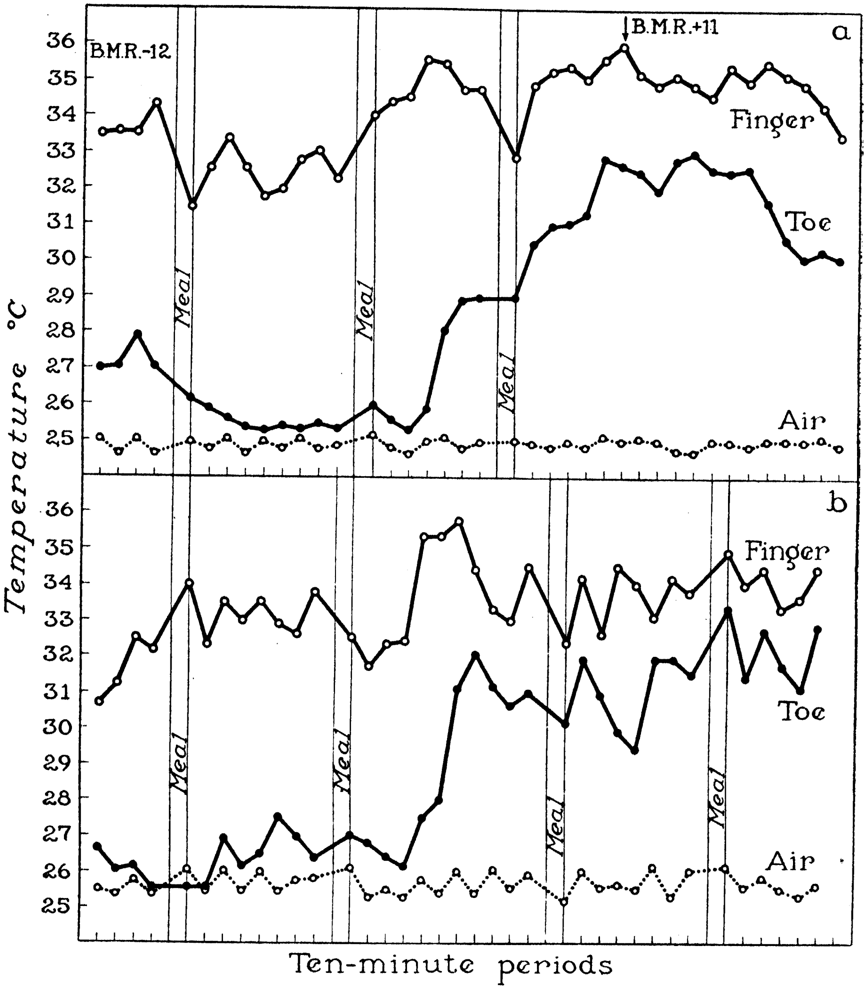

Fig. 3. Comparison of the effect of the ingestion of three meals in close succession at one time and four meals at another time on the skin temperature of the extremities of one subject. Slight sweating was present after the third meal in both observations and after the fourth meal also.

The blood pressure and pulse rate were observed at various intervals before and after the ingestion of food. Likewise, the presence of sweating on the body, particularly of the extremities, was noted before and after the ingestion of the various meals.

RESULTS

Fifty-two observations of skin temperature of the extremities, blood pressure, pulse rate and mouth temperature were made on the 24 normal subjects. Seventeen were made before and after the ingestion of only one meal, one before and after ingestion of two meals, 27 before and after ingestion of three meals and 7 of four meals.

The average response of the skin temperature to three successive meals is shown in figure 1. The skin temperature of the fingers increased 4°C. and that of the toes increased 6°C. The vasodilatation began after the first meal and was maintained for five hours, when the observations were stopped. The feet remained dry and the metabolic rate increased from −8 per cent during the control period (basal) to +26 per cent.

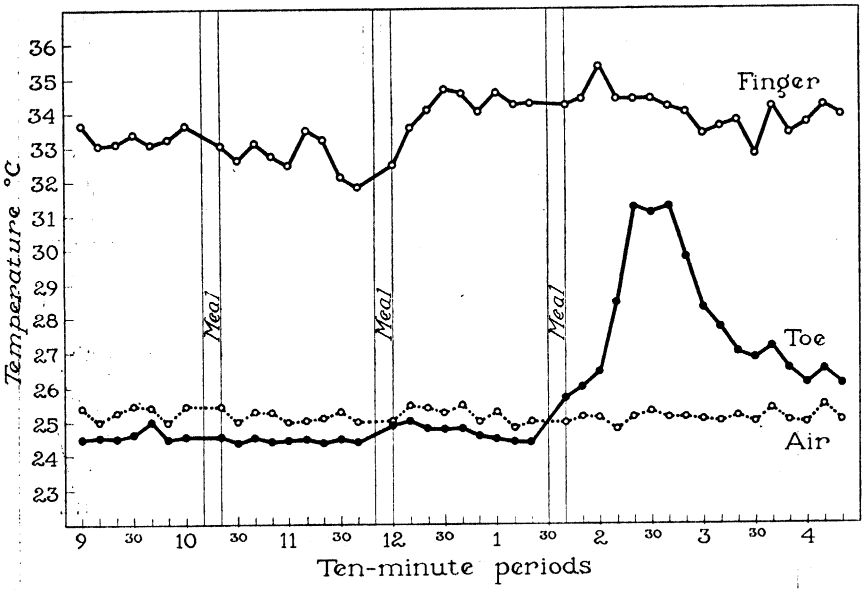

FIG. 4. EFFECT OF PROFUSE SWEATING on the feet of a normal individual. No increase in the skin temperature of the toes occurred until after the third meal and then was maintained at its peak only for 30 minutes.

The response of the skin temperature of the fingers and toes of a subject with a higher basal metabolic rate to the ingestion of four meals is shown in figure 2. The control skin temperatures of this subject were much higher than those of the subject whose curve was shown in figure 1 and the increase was limited. The increase in the skin temperature of the fingers was only 1°C. and that of the toes was 2°C. Vasodilation was present throughout the period of observation in spite of some sweating.

The comparison of the effect of three meals and four meals on the skin temperature of the toes and fingers of the same subject is shown in figure 3. In spite of some sweating after it, the additional meal prevented the decrease in skin temperature which occurred after the third meal.

The effect of profuse sweating particularly on the skin temperature of the toes of one subject who had a basal metabolic rate from −14 to −18 per cent is shown in figure 4. No increase occurred in the skin temperature of the toes until after the third meal and then it was maintained at the peak for only 30 minutes.

The blood pressure showed little or no rise immediately after eating, while the average rise in the pulse rate was seven beats per minute. The mouth temperature rose an average of 0.3°C. and the highest rise, which was noted in only one individual, was 0.6°C.

TABLE 1. AVERAGE INCREASE OF SKIN TEMPERATURE AND ITS DURATION AFTER SUCCESSIVE MEALS

MEALS INGESTED	OBSERVATIONS	AVERAGE INCREASE IN SKIN TEMPERATURES, *degrees C.*		DURATION OF VASODILATATION
		Toes	Fingers	
				hours
1	17	4.6 ± 0.7	2.5 ± 0.1	2.4 ± 0.3
2	1	3.6	1.5	2.5
3	27	5.6 ± 0.5	2.7 ± 0.3	3.4 ± 0.2
4	7	5.2 ± 0.3	2.1 ± 0.2	5.0 ± 1.2

TABLE 2. AVERAGE PEAK INCREASE IN SKIN TEMPERATURE IN RELATION TO MEALS INGESTED BEFORE FIRST INCREASE

MEALS INGESTED BEFORE FIRST INCREASE		OBSERVATIONS[1]	AVERAGE INCREASE IN SKIN TEMPERATURES, *degrees C.*		DURATION OF VASODILATATION
			Toes	Fingers	
					hours
1	Total	20	5.6 ± 0.8	2.0 ± 0.4	4.0 ± 0.4
	No sweating	12	6.7 ± 0.2	2.4 ± 0.5	4.7 ± 0.2
	Sweating	8	4.1 ± 1.1	1.8 ± 0.7	3.0 ± 0.3
2		12	5.9 ± 0.9	3.5 ± 0.3	2.8 ± 0.3
3		3	6.6	2.8	0.5

[1] Observations made when only one meal was ingested are excluded, so that duration of vasodilatation shows effect of at least one additional meal in each instance.

After the ingestion of one or two meals, the skin temperature of the toes and fingers increased and was maintained for an average of 2 hours and 24 minutes to 2 hours and 30 minutes, while a somewhat greater increase of the skin temperature of the extremities took place and vasodilatation was maintained for 3 hours and 24 minutes to 5 hours after three and four meals (table 1).

When the first rise of the skin temperature of the extremities occurred after the ingestion of the first meal, the rise was more prolonged than when it first occurred after a subsequent meal (table 2). The greatest rise of the skin temperature of the extremities and prolongation of the rise occurred when no sweating was present. When sweating occurred, a decrease in the skin temperature took place. The amount of the decrease was dependent on the degree of sweating.

COMMENT

A complex temperature-regulating mechanism maintains a balance between production and loss of heat. The rate of metabolism after the ingestion of three meals increased to three or four times the basal metabolic rate. As the heat of metabolism is augmented, the balance is maintained at first by loss of heat because of increased vasodilatation, and later this mechanism is supplemented by sweating and increased evaporation. When little or no sweating is present, vasodilatation is greater and is maintained for a longer period of time. When sweating is profuse, heat is lost more rapidly and consequently the skin temperature of the extremities decreases markedly. This decrease is more precipitous the lower the basal metabolic rate.

Vasodilatation which follows the ingestion of food does not take place immediately but only after sufficient absorption of food occurs. The increase of the skin temperatures of the extremities after the absorption of food suggests an increase of blood flow or an increased rate of circulation. Physiologic investigations on animals have substantiated this theory. Herrick, Essex, Mann and Baldes (5) have demonstrated an increase in blood flow in the femoral artery of the dog at the height of digestion of food, and McCracken, Essex and Sheard (6) found a marked shortening of the circulation time between the jugular vein and the femoral artery of dogs at this time.

SUMMARY

Fifty-two observations, including observation of skin temperature of the extremities, blood pressure, pulse rate, mouth temperature and basal metabolic rate in each instance, were made on 24 young normal subjects before and after the ingestion in rather close succession of one to four meals of 500 to 800 calories each.

The rise in the skin temperature of the extremities in the average case was greater and maintained for about an hour longer after the ingestion of three meals than after one or two meals, and an additional two hours of vasodilatation was obtained after the ingestion of four meals in rather close succession. The maintenance of vasodilatation as evidenced by skin temperatures of the extremities was partially dependent on the initial basal metabolic rate and the lack of sweating.

REFERENCES

1. Maddock, W. G. and F. A. Coller. This Journal **106**: 589, 1933.
2. Herrick, J. F. and Charles Sheard. This Journal **113**: 62, 1935.
3. Burton, A. C. and J. R. Murlin. J. Nutrition **9**: 281, 1935.
4. Sheard, Charles. Am. J. Clin. Path. **1**: 209, 1931.
5. Herrick, J. F., H. E. Essex, F. C. Mann and E. J. Baldes. This Journal **108**: 621, 1934.
6. McCracken, E. C., H. E. Essex and Charles Sheard. Am. Heart J. **14**: 60, 1937.

DISTRIBUTION OF ESTRADIOL BETWEEN SERUM AND RED CELLS[1]

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The recent extensive progress in the organic chemistry of the steroids has resulted in a nearly complete picture of the structural relationships of the biologically active steroids and the steroid hormones and has yielded a basis for speculation on the mechanism of steroid hormone action. Yet information regarding the physio-chemical status and distribution in biologic systems, which is necessary before the fate of these substances can be envisaged, is practically nil. The state and distribution should be largely dependent upon physical properties, such as solubility, surface tension, viscosity, etc. The physical chemistry of the steroids is as yet a closed book.

The present report is concerned with the equilibrium regulating the distribution of estradiol between red cells and serum. The biphase system serum-red cells is chosen as a starting point, being convenient and reproducible. In general the distribution of a substance between two phases, barring compound formation, is determined by the solubility and the state of aggregation. The problem has been attacked from this angle. Since the desired solubility data for estradiol were not available, it was first of all necessary to make solubility determinations, before the equilibrium studies could be undertaken. These include the solubility of estradiol at ionic strength of 0.155 and the influence of pH (pH 5.3–8.6) and temperature; the solubility in albumin solution and the influence of pH and temperature; the solubility in globulin and in lecithin; the solubility in serum; and finally the influence of other steroids on the solubility. Having these data, the distribution of estradiol between red cells and either saline, albumin solution or serum was undertaken. This paper omits the problems of the influence of ionic strength, the activity coefficients or the physio-chemical properties of the proteins which influence solubility. This more chemical phase of the work will be published with similar studies upon testosterone and progesterone, so that the results may be more properly correlated.

EXPERIMENTAL

Determination of estradiol. It was originally planned to determine estradiol colorimetrically. Because of the presence of the phenolic group either the diazo-coupling or the Folin-Ciocalteu phenol reaction was available. In the range 0.05 to 0.5 mgm. per 10 cc. volume, the latter reaction followed Beer's law.

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[1] This investigation was aided by a grant from the Donner Foundation, Incorporated, Cancer Research Division.

In order to obtain sufficient amounts of estradiol from saturated aqueous solutions for analytical determinations, it became necessary to concentrate either by evaporation or by extraction. It was found that evaporation at 99°C. produced a loss in hormone and even at room temperature in vacuo at pH 7.0 a variable loss ranging from 37 to 20 per cent was observed. When standards were processed simultaneously the variation in recovery produced a 20 per cent variation in duplicate results. The experience with extraction procedures involving immiscible solvents with quantities as minute as those encountered in our problems has proved unsatisfactory (1). A bioassay procedure for estradiol, which has a standard error of 15 per cent 19 out of 20 times, was therefore developed. The determination of estradiol was performed by this assay (2). This method controls the factor of delayed resorption brought about by certain substances found in biologic fluids, which affects the assay profoundly. The assay test solutions are stable over long periods of time. It was merely necessary to separate solution from solute after equilibration and dilute an adequate sample with saline and sodium lauryl sulfate (S.L.S.) to bring about a final concentration of 0.5 per cent S.L.S. This solution was kept refrigerated until used for the bioassay. On standing one year, the standard so prepared lost 11 per cent of its activity, when compared with a new standard. The advantages of an accurate bioassay over a colorimetric method were revealed in work with the determination of testosterone and progesterone by the Zimmermann reaction. It was found that each change of medium presented a minor research problem as to control of the blank and recovery, and reaction products with chromogenic properties could not be differentiated from the original hormones. In the present studies a reaction product of estradiol would possibly be an oxidation to estrone or estriol, both of which have such comparatively low biologic activity that any such conversion to any appreciable extent would have been detected in the balance sheet.

Before experimenting with rabbit red cells and serum it was necessary to determine whether there were present amounts of estrogenic substances sufficient to interfere with the levels used in our experiments. A composite sample of defibrinated bloods from six female rabbits was diluted 1 to 7 with isotonic saline; 0.5 per cent S.L.S. was added. This solution was administered to immature female rats according to the standard assay procedure. In eight rats the mean uterine weight was 16.5 ± 0.7 mgm., which is the value for the control uterine weight. The amount of serum and red cells administered was four times the amount present in any of the assay solutions.

The alpha estradiol used in these experiments had a melting point of 176.8 to 177.1°C. The crystalline bovine albumin and electrophoretically homogeneous gamma globulin were products of the Armour Research Laboratories. The lecithin used was the product of Wilson and Company. Equilibration in the solubility studies was performed in Pyrex glassware with tin-foil covered or ground glass stoppers. The concentration of the estradiol in the solid phase was such that more than 90 per cent remained after equilibrium was attained. Aseptic technique was used in the preparation of glassware and solutions. The

TABLE 1. THE SOLUBILITY OF ALPHA ESTRADIOL IN AQUEOUS SALINE, ALBUMIN, GLOBULIN OR LECITHIN SOLUTIONS AND IN RABBIT SERUM

SOLVENT	TEMP.	pH	EQUILIBRATION TIME	UTERINE WT. FOR 0.15 γ STD.	BIOASSAY DILUTION OF UNKNOWN	UTERINE WT. FOR UNKNOWN	SOLUBILITY IN MOLES PER LITER
	°C.		hrs.	mgm.		mgm.	
NaCl – phosphate $\mu = .155$	37.5	7.0	70	64 ± 1.8	16.7	64 ± 3.1	$(9.2 \pm 0.8) \times 10^{-6}$
			140	63 ± 2.0	16.7	65 ± 1.7	$(9.7 \pm 0.5) \times 10^{-6}$
			210	58 ± 2.0	16.7	57 ± 1.5	$(9.1 \pm 0.5) \times 10^{-6}$
NaCl – phosphate $\mu = .155$	37.5	8.6	70	63 ± 2.2	33.3	39 ± 0.8	$(8.5 \pm 0.6) \times 10^{-6}$
			140	62 ± 3.2	33.3	38 ± 2.3	$(8.8 \pm 0.7) \times 10^{-6}$
			210	65 ± 2.7	33.3	41 ± 1.8	$(8.8 \pm 0.6) \times 10^{-6}$
NaCl – phosphate $\mu = .155$	25.0	8.5	70	61 ± 4.4	16.7	49 ± 4.1	$(6.6 \pm 0.4) \times 10^{-6}$
			140	62 ± 3.9	16.7	49 ± 3.1	$(6.5 \pm 0.45) \times 10^{-6}$
			170	58 ± 3.1	16.7	47 ± 3.6	$(6.7 \pm 0.5) \times 10^{-6}$
1% albumin, 0.8% NaCl	37.5	5.5	24	67 ± 3.0	167	62 ± 4.0	$(8.1 \pm 0.7) \times 10^{-5}$
			50	61 ± 2.5	167	46 ± 2.2	$(6.1 \pm 0.2) \times 10^{-5}$
3% albumin, 0.8% NaCl	37.5	5.5	24	61 ± 3.0	500	41 ± 1.4	$(1.6 \pm 0.15) \times 10^{-4}$
			71	61 ± 2.7	500	48 ± 1.5	$(2.0 \pm 0.15) \times 10^{-4}$
3% albumin phosphate – NaCl $\mu = .155$	37.5	6.9	48	74 ± 2.8	500	51 ± 4.5	$(1.6 \pm 0.15) \times 10^{-4}$
			73	69 ± 2.6	500	53 ± 1.3	$(2.0 \pm 0.08) \times 10^{-4}$
3% albumin, 0.8% NaCl	25.0	5.5	43	70 ± 2.5	500	31 ± 1.7	$(8.3 \pm 0.5) \times 10^{-5}$
			71	71 ± 2.3	500	31 ± 1.7	$(8.1 \pm 0.8) \times 10^{-5}$
Cholesterol – 3% albumin $\mu = .155$	37.5	5.5	48	62 ± 4.0	500	41 ± 2.5	$(1.5 \pm 0.1) \times 10^{-4}$
Progesterone – 3% albumin $\mu = .155$	37.5	5.3	74	55 ± 2.4	500	35 ± 1.6	$(1.4 \pm 0.1) \times 10^{-4}$
Testosterone – 3% albumin $\mu = .155$	37.5	5.3	74	55 ± 1.7	500	30 ± 1.4	$(1.0 \pm 0.08) \times 10^{-4}$
Serum 6.9% protein	37.5	8.3	49	65 ± 4.7	1000	54 ± 3.6	$(4.3 \pm 0.3) \times 10^{-4}$
0.19% lecithin, 0.9% NaCl	37.5	6.0	75	64 ± 3.3	66.7	68 ± 2.8	$(4.0 \pm 0.2) \times 10^{-5}$
		6.2	146	56 ± 2.9	66.7	53 ± 3.3	$(3.4 \pm 0.2) \times 10^{-5}$
1% globulin, 0.8% NaCl	37.5	6.5	24	62 ± 1.2	167	36 ± 1.6	$(3.9 \pm 0.3) \times 10^{-5}$
			48	62 ± 1.2	167	28 ± 1.5	$(2.6 \pm 0.2) \times 10^{-5}$

equilibration solutions were agitated in thermostatically controlled cabinets in the day and were kept in water thermostats at night; in these, agitation was brought about by eddy currents formed by the stirrer. Filtration was performed in a thermostatically controlled cabinet. The melting points of the residues obtained from equilibration at ionic strength 0.155 were not lowered, indicating freedom from decomposition.

The details of the experiments in which red cells were equilibrated with the various aqueous phases are described under results. These experiments were not performed under aseptic conditions. The importance of avoiding hemolysis during the experiment is obvious. Except in the experiments dealing with serum, hemolysis could not be completely avoided. The degree of hemolysis was measured colorimetrically when it occurred. In general it was considerably less than 0.2 per cent. Some volume changes in the cells occurred during equilibration. These were measured, but were of a magnitude less than that of the error of the bioassay, so no correction was made in the calculations.

TABLE 2. DISTRIBUTION OF ESTRADIOL BETWEEN BIPHASE SYSTEMS

EXP. NO.	PHASE A BEFORE EQUILIBRATION		PHASE B BEFORE EQUILIBRATION		TOTAL CONCENTRATION ESTRADIOL IN GAMMA				$\frac{\text{C CELLS (B)}}{C_A}$
	Volume and composition	gamma estradiol/cc.	Volume and composition	gamma estradiol/cc.	A before	A after	B before	B after	
1	30 cc. 0.9% NaCl	2.0	6 cc. washed red cells	—	60.0	18.5	—	(41.5)	11.2
2	24 cc. 0.9% NaCl, 6 cc. serum	1.6	6 cc. washed red cells	—	43.2	38.4	—	(4.6)	0.60
3	30 cc. 0.9% NaCl	1.6	6 cc. washed red cells	—	43.2	14.4	—	(28.8)	10.0
4	15 cc. 0.9% NaCl	—	3 cc. red cells above, after	4.8	—	4.2	(14.4)	(10.2)	12.2
5	6 cc. serum	4.16	6 cc. red cells	—	28.4	23.1	—	5.4 (5.3)	0.23
6	5 cc. serum	—	5 cc. red cells saline estradiol treated	(6.2)	—	26.6	(31.1)	4.5 (4.5)	0.17
7	5 cc. serum	4.0	5 gm. minced uterus	—	21.5	16.7	—	(4.8)	0.29
8	5 cc. serum	4.0	5 gm. minced thymus	—	21.5	10.4	—	(11.1)	1.07
9	6 cc. 3% albumin, 0.8% NaCl	4.16	6 cc. washed red cells	—	26.3	19.4	—	(6.9)	0.35
10	6 cc. 3% albumin, 0.8% NaCl	4.16	6 cc. sesame oil	—	26.3	<2.0	—	>(24.6)	
11	3 cc. 3% albumin, 0.8% NaCl, pH 5.5	44	2 sq. inch collodion	—	132	58	—	(74.0)	

RESULTS

Solubility determinations of estradiol at ionic strength 0.155 in aqueous sodium chloride-sodium phosphate solutions were made at pH 7.2 and pH 8.6

at 37.5°C. and at pH 8.5 at 25.0°C. It is apparent that equilibrium is reached within 70 hours, since subsequent samples at 140 hours and as late as 210 hours are in excellent agreement with the samples taken at 70 hours. In the range studied pH was without measurable influence. From these data, the heat of solution is calculated by the Van't Hoff isochore equation as 4300 calories per mole.

$$\Delta \text{ Heat solution} = \frac{4.6 \log \frac{8.8}{6.6}}{\frac{1}{298.1} - \frac{1}{310.6}} = 4300 \text{ calories per mole}$$

Solubility determinations of estradiol in 1 and 3 per cent crystalline plasma bovine albumin solutions at pH 5.5 and ionic strength of approximately 0.155 were made at 25.0°C. and 37.5°C. A 3 per cent albumin concentration at pH 6.9 was also studied. Solubility was determined in a 1 per cent solution of electrophoretically homogeneous bovine gamma globulin at pH 6.5 and 37.5°C. A comparison of the data for the latter compound shows a significant fall in solubility at 48 hours of equilibration from the value of 3.9×10^{-5} moles per liter obtained at 24 hours. It was noticed that during the period of equilibration the globulin solution developed an opacity, indicating denaturation. This was not observed in the case of the albumin experiments. At 25°C. equilibrium in the albumin experiments was reached at 43 hours. The mechanical agitation at 25°C. was more vigorous than that used at 37.5°C. In the latter experiments the solubility apparently increased slightly on further equilibration, but the difference approaches the experimental error. In the range pH 5.3–6.9, pH is without effect upon solubility in albumin.

The heat of dispersion of estradiol by albumin may be calculated from the solubility data at 25.0°C. and 37.5°C. after subtracting the respective solubilities in saline solution of the same ionic strength.

$$\text{Heat of dispersion} = \frac{4.6 \log (2.0 - 0.1/0.82 - .07)}{\frac{1}{298.1} - \frac{1}{310.6}} = 13{,}700 \text{ calories per mole}$$

The solubility data in 1 and 3 per cent albumin solution indicate a proportionality between estradiol dispersed and amount of protein acting as the dispersing agent, viz., $(7.1 - .9)\,3 = 18.6$ versus $20.0 - .9 = 19.1$. In 1 and 3 per cent albumin solutions, respectively, 7.1 and 20.0 are the solubilities, and 0.9 is the solubility in isotonic saline.

Influence of another sterol. The influence of cholesterol, testosterone or progesterone upon the solubility of estradiol in 3 per cent bovine albumin solution at 37.5°C. and pH 5.3–5.5 was studied in three experiments. A large excess of the steroid or sterol was used as the solid phase and the time of equilibration was of the same duration as that chosen for the solubility determination of estradiol in 3 per cent bovine albumin. The testosterone and progesterone were determined by a modification of the Zimmermann reaction. The values obtained, 11.8×10^{-4} moles per liter for testosterone and 3.8×10^{-4} moles per liter for progesterone, are in excellent agreement with unpublished data in this laboratory on the solubility of these steroids in 3 per cent bovine albumin, at the same temperature and pH. In contrast, the solubility of estradiol in the presence of testosterone fell to $(1.03 \pm .08) \times 10^{-4}$ from $(2.0 \pm 0.15) \times 10^{-4}$, which is the value obtained in the absence of testosterone. The value for estradiol in the presence of progesterone, $(1.4 \pm 0.11) \times 10^{-4}$, is significantly less than the solubility obtained in the absence of this hormone. While the value obtained in the presence of cholesterol $(1.5 \pm 0.1) \times 10^{-4}$ is less, the difference is not significant. An attempt to estimate by the Liebermann reaction the amount of cholesterol dispersed by the albumin was unsuccessful because of the low solubility. It was, however, established that the amount was less than 5×10^{-5} moles per liter.

Coefficient of distribution. The distribution of estradiol between red cells (or minced thymus or minced uterus) and either isotonic NaCl solution, 3 per cent bovine albumin solution or rabbit serum was studied in the ten experiments recorded in table 2. The estradiol was dissolved in the saline, albumin solution or serum by adding an alcoholic solution of estradiol from a serological pipette, so that the final concentration of alcohol was less than 0.5 per cent and the amount of estradiol was below the solubility as determined in previous experiments. These solutions were then equilibrated for 10 minutes at 37.5°C. with rabbit red cells which had been washed five times with isotonic saline. The volumes for the two phases are given in table 2. After equilibration the red cells were removed by centrifugation. Bioassays were performed before and after equilibration. The bioassays for the solute before equilibration agreed with the amounts added within 0, 11, 11, 12, 7, 7 and 5 per cent. In most cases the amount of estradiol taken up by the red cells was determined by difference (figures in parentheses). In two experiments assays of the red cells were performed and agreed exactly with theory. Assay of the material in red cells is feasible if the solution can be assayed within a few days after the experiment is performed, but is not feasible if the solution stands for any length of time as precipitation occurs.

Experiments 1, 3 and 4 are concerned with the distribution between saline and cells. The coefficients of distribution C cells/C saline in the three experiments, viz., 11.2, 10.0 and 12.2, are in good agreement. In experiment 4, an aliquot of the cells after equilibration in experiment 3 was re-equilibrated with saline, demonstrating the return of estradiol from the cells to the saline. Experiments 5 and 6 are concerned with the equilibrium between rabbit serum and red cells, experiment 6 demonstrating the return of estradiol from the cells to the serum. These experiments were performed under alveolar CO_2 tension. In experiment 5, the red cells were not washed in saline but packed and separated from their original serum and then equilibrated with serum to which estradiol had been added. Experiments 7 and 8 must be regarded as exploratory, since the minced tissues are not made up of one type of cell and the presence of fat probably has a profound influence as demonstrated in experiment 10. Experiment 9 gives the distribution for 3 per cent bovine albumin. In experiment 11, a solution of 3 per cent albumin saturated with estradiol at 37.5°C. was equilibrated with two square inches of collodion for one hour under agitation in a mechanical shaker at 37.5°C. Over half the estradiol was removed from solution.

DISCUSSION

It is at once apparent that the 50-fold change in the distribution coefficients when serum is substituted for saline may be relegated to a large degree to the influence of albumin. In order to throw some light on the nature of the distribution, the data may be treated as a biphase system in which the red cells become one of the phases. The boundary of the cell is regarded as continuous with the rest of the cell. The distribution coefficient should then be equal to the ratio of the solubility of estradiol in cells and in the other phase if the assumption is made that there is no change in activity coefficients or state of aggregation, and no compound formation. Using the solubility data for saline, albumin or serum, the solubility in red cells becomes:

$9.0 \times 10^{-6} \times 10.50 = 9.5 \times 10^{-5}$ (as calculated from saline data)
$2.0 \times 10^{-4} \times 0.35 = 7.0 \times 10^{-5}$ (as calculated from albumin data)
$4.3 \times 10^{-4} \times 0.20 = 8.6 \times 10^{-5}$ (as calculated from serum data)

The agreement is striking and well within the experimental error. The conclusion to be reached is that the passage of estradiol from serum to red cell follows the laws of solubility, there being no indication of specific compound formation. Albumin has a marked influence on the solubility of estradiol and therefore a marked influence upon withholding its passage into the red cell. The removal of estradiol from an albumin solution by red cells, sesame oil or collodion indicates there is no compound formation. Among the constituents of serum which contribute to the dispersion of estradiol, roughly three fourths of the effect can be accounted for by the albumin fraction, another 10% by lecithin and the remainder by the globulin fraction. On a weight basis lecithin has approximately three times the effect of albumin. The remarkable affinity which serum albumin has for small or large ions, or even polar compounds, has recently been stressed by Cohn *et al.* (4). The profound effect of serum albumin in dispersing estradiol is therefore not a unique property. Since the lecithin content of serum and red cells is nearly the same, the influence of lecithin in the equilibrium is balanced. However, in other cells in which the lecithin content is high, its influence may be of physiologic importance as a factor in concentrating or localizing the hormone. The extraction of estradiol by fat as a physiologic process will depend on whether or not the membrane of the fat cell is permeable. These are problems reserved for further study.

Two of the observations made in these studies may have a direct bearing on the mechanism of physiologic activity. One of these is the blocking of the dispersion of estradiol in albumin solution by testosterone. This is probably a competition between the secondary valence bonds of testosterone and estradiol for those of albumin. On the other hand the block by progesterone is less.

There is therefore a rough correlation between physiologic action and physiochemical status. It must be admitted that such a correlation may be fortuitous. The other observation is the relatively high heat of dispersion of estradiol in comparison to that of the other hormones (4). Estradiol dispersion is much more sensitive to temperature change than are the other hormones. Other factors being equal, the testis would have a lessened ability to concentrate estradiol, since it is at a lower temperature.

SUMMARY

The solubility of estradiol in aqueous solution at 37.5°C. and ionic strength of 0.155 is $(9.0 \pm 0.3) \times 10^{-6}$ moles per liter. A shift of pH from 7.0 to 8.6 is without influence, indicating complete hydrolysis and the absence of salt formation with the weakly acid phenolic group. The heat of solution of estradiol in water in the temperature range 25.0°C. to 37.5°C., at ionic strength 0.155, is 4300 calories per mole.

The solubility of estradiol in 3 per cent bovine albumin at 37.5°C., ionic strength 0.155 and pH range 5.5 to 6.9 is $(2.0 \pm 0.1) \times 10^{-4}$ moles per liter. Correcting for solubility in water, dispersing power of bovine albumin toward estradiol is one mole estradiol per 2.2 moles albumin. The heat of dispersion in 3 per cent albumin is 13,700 calories per mole. The solubility of estradiol in rabbit serum may be roughly apportioned to the following constituents: albumin 75 per cent, globulin 15 per cent and lecithin 10 per cent.

The distribution coefficients of estradiol between red cells and either saline, albumin solution or serum are inversely proportional to the solubilities of estradiol in these solutes. On re-equilibration of red cells, which have extracted estradiol from saline or serum solution, with the same respective solutes, the distribution coefficient remains the same. Estradiol is removed from albumin solution by collodion or by sesame oil.

The experiments demonstrate rather conclusively that the distribution of estradiol in blood is governed solely by the forces which govern its solubility. There is no indication of compound formation or inactivation. To it, the red cell is freely permeable. The solubility of estradiol in albumin solution is markedly lessened by competition with testosterone. The effect of progesterone under similar conditions is less than that of testosterone.

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REFERENCES

1. Hawk, P. B., B. L. Oser and W. H. Summerson. Practical Physiological Chemistry, 12th Edition. The Blakiston Company, 1947.
2. Bischoff, F. and H. R. Pilhorn. This Journal **150**: 444, 1947.
3. Cohn, E. J., W. L. Hughes Jr. and J. H. Weare. J. Am. Chem. Soc. **69**: 1753, 1947.
4. Bischoff, F. and H. R. Pilhorn. Unpublished data.

HEREDITARY OBESITY AND TEMPERATURE REGULATION

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In the course of studies on the regulation of body temperature in the normal white mouse (1) it was noted that the amount of body surface exposed to the environment and the activity were two dominant features in such regulation. The hereditary obese yellow mouse, described by Danforth (2, 3), would appear to be a favorable experimental animal for clarification of this problem for several reasons. First, thin normal controls with the same genetic constitution as the obese mice are available (3); second, the disproportionately great heat loss afforded by the large surface area of the obese mice might be expected seriously to handicap their temperature regulation at low environmental temperatures; third, no surgical or other experimental procedures are necessary to produce the obesity; and finally as Danforth (3) states, "Adiposity is of further interest since it furnishes readily available material to illustrate the interaction of hereditary and environmental factors in the production of a hereditary trait". In view of the rôle of the hypothalamus in temperature regulation (4) and in experimental and clinical obesity (5), it would appear likely that a comparison of the adequacy of regulation of body temperature in obese and thin controls would aid in the analysis of these two rôles of the hypothalamus. The present study was undertaken to compare the body temperature responses of obese and normal mice of the same genetic strain at various environmental temperatures. It was made possible by the use of mice kindly provided by Dr. C. H. Danforth of the Department of Anatomy.

MATERIAL AND METHODS

Two groups of six animals each were used. *Group I*, which was composed of thin normal yellow mice, had a mean body weight, during the course of the experiments, from 29.3 to 31.9 grams. *Group II*, which contained obese yellow mice genetically identical with those in *Group I*, had a mean body weight of 49.6 to 52.9 grams. These two groups were kept under the same laboratory conditions, allowed an abundance of mixed grains, lettuce and fortified margarine; they were treated in an identical fashion in all experiments.

The adequacy of regulation of body temperature in these two groups was assessed at environmental temperatures from 5° to 40°C. Each experiment consisted first of determining the rectal temperature of each mouse at room temperature by means of a potentiometer and a thermocouple inserted to a constant depth (1. 4 mm.); the animals were weighed and placed in jars similar in design

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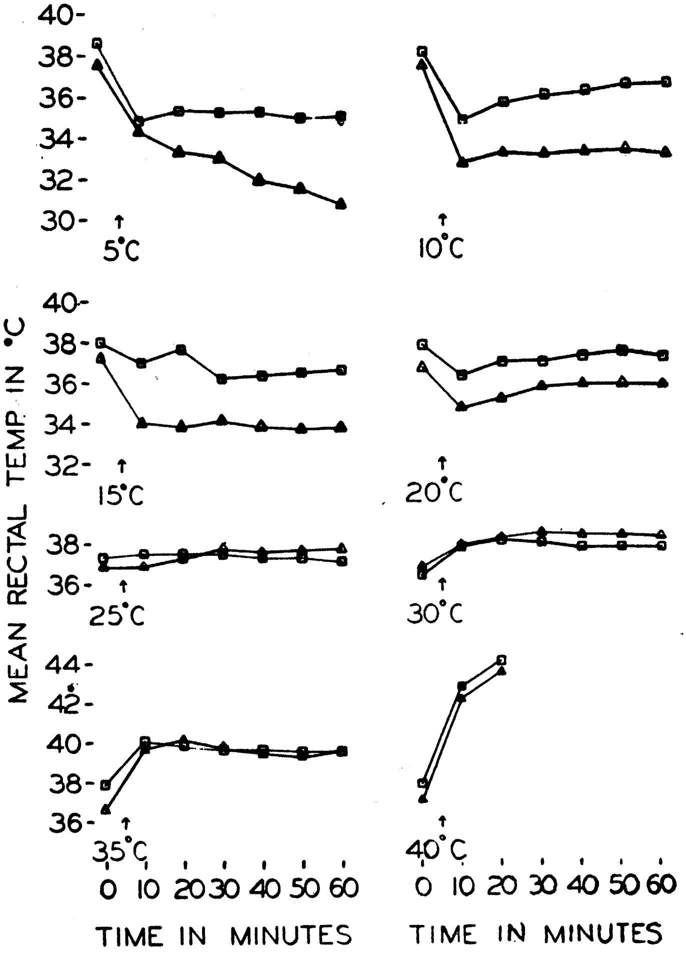
[1] Aided by the technical assistance of G. J. Fuhrman and by a grant from the Fluid Research Fund, Stanford University School of Medicine.

to those described by Fuhrman, McLin and Turner (6). The containers were placed in a thermostat and the temperature of each jar and mouse recorded at 10-minute intervals for one hour. The air temperature in and above the jars was maintained within one degree of that of the thermostat. The experimental environmental temperatures, in the order in which they were utilized in separate experiments, were 25°, 20°, 15°, 10°, 5°, 25°, and 40°C.



FIGS. 1–8. TIME COURSE OF MEAN RECTAL TEMPERATURE of obese and normal mice of the same strain at environmental temperatures from 5°–40°C. Normal mice are represented by squares; obese, by triangles.

The experiment, at each environmental temperature used, was repeated alternately on the two groups on the following or subsequent day. All experiments were conducted in the morning to minimize any differences in body temperature values determined at different times during the day (cf. 6). The thermostat was well illuminated in order to minimize the activity of the animals (7).

A scale of observable activity was employed to estimate the amount of spontaneous activity of the individual mice during the course of adaptation to the various environmental temperatures. Values from 1 to 3 were used: (1) for mice which were quiet, resting and awake; (2) for mice exhibiting movement in position; (3) for mice moving about the container.

TABLE 1. EFFECT OF ENVIRONMENTAL TEMPERATURES ON RECTAL TEMPERATURE

ENVIRONMENTAL TEMPERATURE	MEAN RECTAL TEMPERATURE AT ONE HOUR		P VALUE
	Group I	Group II	
°C.	°C.	°C.	
5	34.97	30.73	<0.0001
10	36.56	33.12	<0.0001
15	36.52	33.76	<0.0001
20	37.15	35.87	0.40
25	37.10	37.75	<0.0001
30	37.80	38.31	0.40
35	39.63	39.57	<0.0001
40	44.07	43.57	

TABLE 2. MEAN RECTAL TEMPERATURE AT ROOM TEMPERATURE

	n	$\bar{x}$	RANGE	S.E.$_{\bar{x}}$	$P_{\bar{x}}$
Normal white mice (Fuhrman, 1943)......	100	36.81	34.0–38.3	0.026	
					<0.0001
Group I (Thin yellow mice)................	96	37.69	33.5–39.2	0.092	
					<0.0001
Group II (Obese yellow mice)................	96	37.00	35.0–38.3	0.035	

TABLE 3. EFFECT OF ENVIRONMENTAL TEMPERATURE ON ACTIVITY

ENVIRONMENTAL TEMPERATURE	MEAN ACTIVITY		P VALUE
	Group I	Group II	
°C			
5	2.57	2.38	
10	2.32	1.77	
15	2.43	1.83	
20	2.83	1.93	
25	2.10	1.71	
30	2.17	1.89	
35	2.10	1.97	
			<0.0001

RESULTS

The effects of the environmental temperatures used in these experiments on the mean rectal temperatures of thin yellow mice (*Group I*) and genetically identical obese yellow mice (*Group II*) are presented in figures 1–8. Figures 1–4, 6 and 7 represent mean values for two experiments at each environmental temperature; figure 5 shows the results of four experiments at 25°C. Figure 8 demonstrates the effect of 40°C. on the mean rectal temperature of the two groups for a 20-minute period at which time the animals were dead or moribund. It is interesting to note that in *Group I* the mean body temperature at which death occurred was 44.06°C.; in *Group II* the lethal temperature was 43.57°C.

The observed differences in response between the two groups of mice over the one-hour period were highly significant (see table 1) in the experiments conducted at 5°, 10°, 15°, 25°, and 35°C. The P values were <0.0001, i.e. such differences would be expected to occur once in 10,000 times by chance. The differences in response at 20° and 30°C. were not significant. The method used was that of Fisher (8).

Table 2 presents the mean, range, standard error of the means, and the probability of differences between the means of rectal temperatures of three groups of mice at room temperature. Values for *Groups I* and *II* were obtained at the zero time of each individual experiment; values for normal white mice were obtained from Fuhrman (9). The differences between means are highly significant, i.e. such differences would be attributable to chance once in 10,000 times.

Table 3 visualizes the mean spontaneous activity of each group (as estimated on the scale 1–3) over the one-hour experimental period at each environmental temperature employed. The standard error of the mean differences in response was 0.01, and the probability of such differences occurring due to chance alone is less than once in 10,000 times.

DISCUSSION

Rectal temperature in the normal white mouse remains fairly constant over a narrow range of environmental temperature, i.e. between 20° and 30°C. (cf. 10). The adjustment of the thin yellow mice used in this study is similar in this respect to normal white mice, i.e. the body temperature was constant between 20° and 30°C., but at a higher level. However, the obese mice show a less adequate adjustment; the body temperature varies almost directly with the environmental temperature (see figure 9). This relationship is similar to that found in amphibia and reptiles (11). The differences in adjustment at the various environmental temperatures between the thin and obese mice may perhaps be explained best by the differences in activity and the differences in the amount of surface exposed.

At the low environmental temperatures, 5°–15°C., the thin mice were more active (see table 3) and hence not only directly produce more heat, but indirectly conserve heat by moving about on the copper mesh. In contrast, the obese mice move about to a lesser extent and secondarily lose considerable heat from the large ventral surface exposed to the copper mesh platforms on which they are resting. The insulation afforded by the subcutaneous fat (3) is apparently ineffective in reducing such heat loss to any appreciable extent.

At room temperature (18°–20°C.) both groups are well below the critical temperature for the mouse (10, 12, 13), but the thin mice are able to maintain their body temperature by increasing their motor activity, whereas the sluggish obese mice lose more heat than they produce under these conditions; as a result their body temperature falls (see figure 9 and table 3).

At the high environmental temperatures (30°–40°C.) all the mice showed a rise in rectal temperature in spite of the facilitation of heat loss by the spreading of saliva over their coats (cf. 10, 14). Since the obese group produces less heat by reason of the lower level of activity and since their heat loss is greater than that of the thin group, a lower body temperature would be expected but is found only during the course of the hour exposure at 30°C. Apparently, the lack of

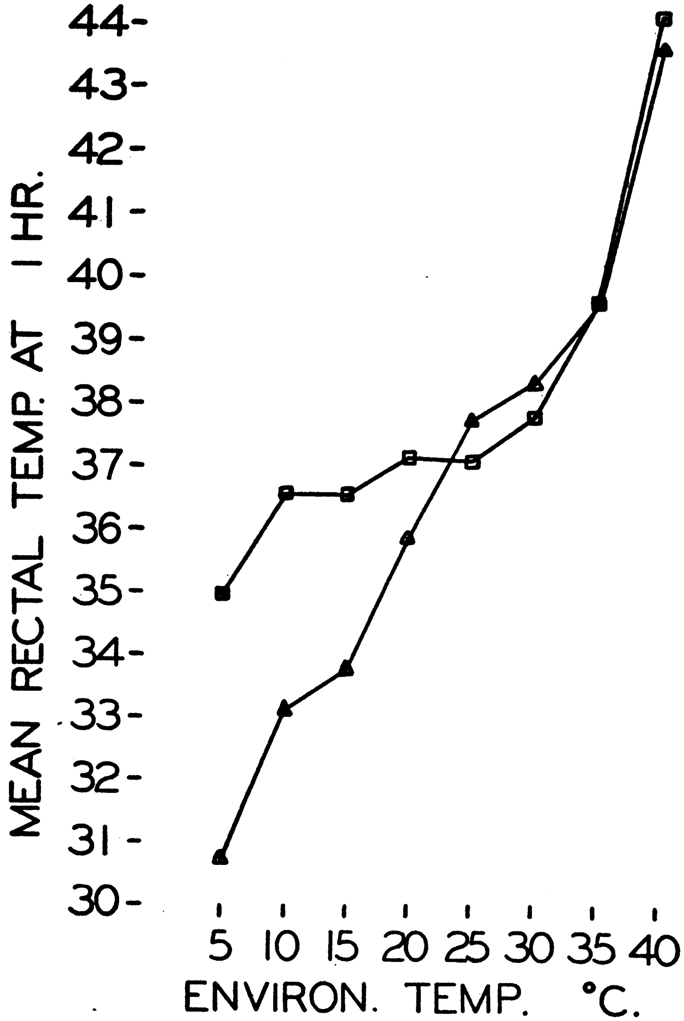


FIG. 9. MEAN RECTAL TEMPERATURE of obese and normal mice at the end of one-hour exposure to environmental temperatures from 5°–40°C.

true physiological regulation by evaporation (10) is the cause of the body temperature rise in all mice.

Were the ultimate physiological cause of hereditary obesity known, speculation as to the differences in regulation of body temperature in obesity would be more profitable than at the present time. If deficient thyroid secretion were responsible for the depressed activity (15, 16) and the resulting decreased rectal temperature, cytological evidence of depressed function might be expected (17). Weitze (18) has observed no differences in the appearance of the thyroids taken from yellow obese and from normal mice of the same strain. However, no specific cytological stains were used and no Golgi preparations made. Further, if the thyroid were directly responsible, the basal metabolism of the obese mice would be expected to be lower than that of normal animals. Though basal metabolic rates of obese and thin yellow mice are not at present available, calculation of Rytand's data (19) obtained at room temperature reveals no significant difference in oxygen consumption of obese and thin yellow mice. On the basis of Q_{O_2} (cubic centimeters of oxygen per gram per hour), however, the obese mice have a metabolic rate significantly below that of the thin control animals. The mean Q_{O_2} for obese mice is 3.45 ± 0.008; for thin mice, 5.29 ± 0.22. The P value is <0.0001.

Dickerson and Gowen (20) also noted a depressed activity in obese mice and state, "... the yellow gene ... produces obesity, primarily by increasing the food intake and by reducing energy expended especially for activity. Both of these effects increase the energy available for storage as fat, whereas the first raises only slightly, and the second reduces, the energy dissipated in body work." It is possible that the disturbed temperature regulation in the obese mice noted here is one of the manifestations of "deficiencies in the regulation of energy intake and energy output" which Brobeck (5) states as a probable cause of experimental obesity produced by hypothalamic lesions.

Though there is at the present time no anatomical evidence of localized lesions of the hypothalamus in the yellow obese mice, there is some indirect physiological evidence linking hypothalamic function with hereditary obesity. First, hyperphagia and resulting obesity are common signs of surgically produced hypothalamic lesions (21, 22). These signs also occur in this strain of yellow obese mice (18, 20, 23, 24). Decreased sensitivity to insulin has been reported both in animals with hypothalamic lesions (21) and in obese yellow mice (18). Further, Hetherington and Ranson (22) and others (25, 26, 27) found that spontaneous activity of animals with posterior hypothalamic lesions is depressed, as is the activity of yellow obese mice (see table 3). Dickerson and Gowen (20) also report such depressed activity in these mice. Finally, Ranson (4) observed the disturbance of temperature regulation afforded by hypothalamic lesions and stated specifically, "Laterally placed moderate-sized lesions in posterior part of hypothalamus cause very great impairment in the ability to regulate against both heat and cold", a finding similar to that herein reported on yellow obese mice.

In view of the functional similarities between experimentally produced hypothalamic obesity and hereditary obesity, it would appear likely that the yellow gene responsible for adiposity in these mice is concerned in some way with hypothalamic integrity. The disorder in carbohydrate metabolism (18), the depressed activity (reported here; Dickerson and Gowen, 20), the hyperphagia (18, 20, 23, 24), the efficiency of food utilization (20), the disturbed temperature regulation (reported here) and even the obesity itself can at the present time perhaps be best explained by a disturbed hypothalamic function.

SUMMARY

1. At room temperature (18°–20°C.) the mean body temperature of genetically obese yellow mice is lower than that of thin yellow mice of the same strain. The rectal temperature of all yellow mice is above that of normal white mice.

2. At an environmental temperature of 25°C. the rectal temperature of both normal and obese yellow mice is constant at the same level.

3. There is evidence of disturbed temperature regulation in this form of hereditary obesity. At low environmental temperatures (5°–20°C.), the mean rectal temperature of obese mice continued to fall during the one-hour exposure, while that of the normal yellow mice fell only during the first 10 minutes and remained at a new low level. At higher environmental temperatures (30°–40°C.) all animals showed an elevation of rectal temperature. The obese mice regulated at a somewhat higher level (at an environmental temperature of 30°C.) than normal mice.

4. Physiological evidence is cited which implicates the hypothalamus in this type of hereditary obesity.

REFERENCES

1. TURNER, M. L. Endocrinology **38:** 263, 1946.
2. DANFORTH, C. H. Anat. Record **29:** 354, 1925.
3. DANFORTH, C. H. J. Heredity **18:** 153, 1927.
4. RANSON, S. W. Research Pub. Assoc. for Research Nervous Mental Disease **20:** 342, 1940.
5. BROBECK, J. R. Physiol. Revs. **26:** 541, 1946.
6. FUHRMAN, G. J., E. D. MCLIN AND M. L. TURNER. This Journal **147:** 284, 1946.
7. BROWMAN, L. G. J. Exp. Zoöl. **94:** 477, 1943.
8. FISHER, R. A. Statistical Methods for Research Workers. 6th ed. London: Oliver and Boyd, 1936.
9. FUHRMAN, G. J. The Effect of Cold and Dinitrophenol on the Metabolism and Temperature Regulation in Albino Mice. M. A. Thesis, Stanford University, 1943.
10. HERRINGTON, L. P. This Journal **129:** 123, 1940.
11. KIRK, R. L. AND L. HOGBEN. J. Exp. Biol. **22:** 213, 1946.
12. TERROINE, E. F. AND S. TRAUTMAN. Ann. physiol. physicochem. biol. **3:** 422, 1927.
13. BENEDICT, F. G. Vital Energetics. Washington, D. C.: Carnegie Institution of Washington, 1938.
14. ROBINSON, K. AND D. H. K. LEE. Proc. Roy. Soc. Queensland **53:** 159, 1941.
15. RICHTER, C. P. Endocrinology **17:** 73, 1933.
16. HALL, V. E. AND M. LINDSAY. Endocrinology **22:** 66, 1938.
17. CRAMER, W. AND R. J. LUDFORD. J. Physiol. **61:** 398, 1926.
18. WEITZE, M. Hereditary Adiposity in Mice and the Cause of this Anomaly. København: Store Nordiske Videnskabsboghandel, 1940.
19. RYTAND, D. A. Personal communication, 1947.

20. Dickerson, G. E. and J. W. Gowen. Science **105**: 496, 1947.
21. Tepperman, J., J. R. Brobeck and C. N. H. Long. This Journal **133**: 468, 1941.
22. Hetherington, A. W. and S. W. Ranson. This Journal **136**: 609, 1942.
23. Rytand, D. A. Proc. Soc. Exp. Biol. and Med. **54**: 340, 1943.
24. Conn, J. W. Physiol. Revs. **24**: 31, 1944.
25. Ranson, S. W. and H. W. Magoun. Ergeb. Physiol. exp. Pharmakol. **41**: 56, 1939.
26. Hetherington, A. W. and S. W. Ranson. Anat. Record **78**: 149, 1940.
27. Hetherington, A. W. This Journal **133**: 326, 1941.

PHYSIOLOGIC ICTERUS OF THE NEWBORN[1]

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Normal erythrocyte levels in the circulating blood are maintained by a balance between the destruction and the formation of the red blood corpuscles. That these two forces are balanced delicately is well attested by the fact that the number of red blood cells remains constant under widely variant circumstances. However, before this state of equilibrium is reached, there is an actual imbalance. The human infant is separated from the maternal circulation with more red blood cells and more hemoglobin than is necessary for carrying oxygen after its lungs have begun to function. At birth, the erythrocyte count and the hemoglobin level are higher than in the adult, and within a short time they are lower than in the adult (1–3), despite the presence in many infants of accessory centers of red cell formation. At birth, the serum bilirubin level is approximately one milligram per cent and by the fifth day this level has risen sharply to approximately five milligrams per cent (4, 5). At this time the jaundice is usually apparent in most infants. The hyperbilirubinemia results from an inability of the liver to excrete the products of hemoglobin breakdown at a rate sufficient to cope with their rate of formation. In the next few days the elevated serum bilirubin has diminished and the jaundice subsides rapidly.

The rôle of fat ingestion in the destruction of red blood cells in normal animals (6–9) and in man (10), as well as in some anemic patients (11), is well established. Since the placenta has been assumed to act as a barrier to the passage of fat from the maternal to the fetal circulation, it was felt that the infant's first meal comprised a comparatively high fat diet, resulting in the first lipemia to which the infant had been exposed. That this may be an important factor in icterus neonatorum seems to be borne out by the observations reported here.

METHODS

Blood was aspirated from the umbilical cord vessels of normal infants at normal deliveries. Initially, a small amount of blood was heparinized for hematocrit determinations, cell counts and hemoglobin estimations, but this was later discontinued. Approximately five cubic centimeters of blood was placed in a tube and allowed to coagulate for serum bilirubin estimation. On the fifth day of life, blood was drawn from the external jugular vein (occasionally from the femoral vein or antecubital vein) for the same purposes. Red blood cell counts were made in a routine fashion. Wintrobe tubes were used for duplicate hematocrit determinations. The Evelyn photoelectric colorimeter was used for the determination of hemoglobin (12) and of serum bilirubin (13).

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Helpful suggestions by Dr. Victor E. Johnson are gratefully acknowledged. The cooperation of Drs. William Dieckman and M. Edward Davis of Chicago Lying-In Hospital and of Drs. Harry B. W. Benaron and Beatrice Tucker of the Chicago Memorial Hospital in securing subjects for this study is greatly appreciated.

Infants were used only if their birth weight exceeded five and one-half pounds, and if they had no congenital abnormalities. Otherwise they were unselected, except that two were dropped from the evaluation when they proved to have blood dyscrasias. Infants were divided into four groups: *a*) those fed in a routine fashion, either on breast milk or on a standard formula (containing approximately 3.6 per cent fat); *b*) those fed exclusively on a formula containing approximately 1.8 per cent fat; *c*) those fed exclusively on a formula containing approximately 0.03 per cent fat; and *d*) those fed on a formula containing approximately 5.5 per cent fat. The bottle-fed babies were not allowed to be brought to breast until the five-day sample had been drawn. Water was offered ad lib. and the intake of formula was unrestricted.

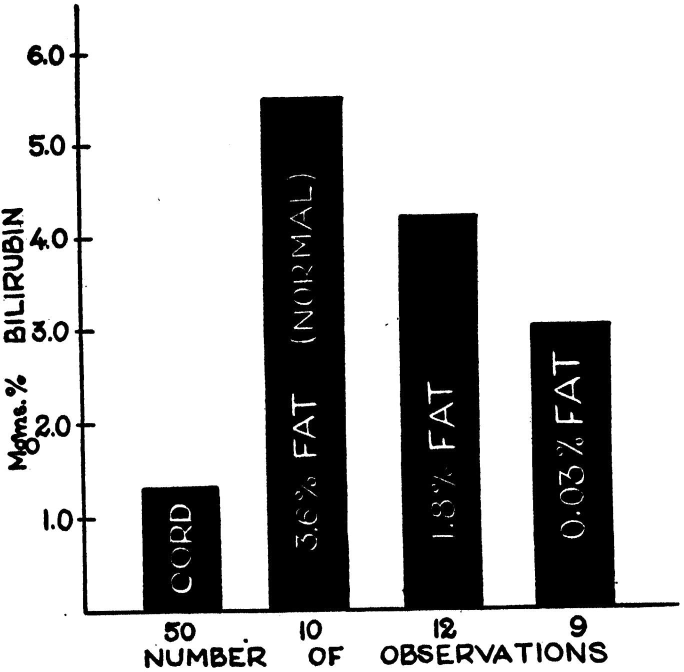

FIG. 1. COMPARISON OF THE AVERAGE SERUM BILIRUBIN LEVEL at birth of all infants studied and the average serum bilirubin level on the fifth day for each of the diets used.

RESULTS

The average serum bilirubin level in 50 cord bloods was found to be 1.36 milligrams per cent. This compares favorably with the level of 1.35 milligrams per cent reported by Waugh *et al.* (4) on 54 babies. Figure 1 shows the average levels on the fifth day of life for the babies on routine feedings, for those on reduced fat intake and for those on a practically fat-free diet. The babies in the group in which the high fat formula was used almost uniformly refused the formula, regurgitated it or took very little, so the findings could not be deemed representative of a high fat intake. In table 1 it can be seen that the range of serum bilirubin levels is wide but no more so in one group than in another. A small group such as this does not lend itself well to statistical analysis, but the averages clearly bear out a definite difference in the level of serum bilirubin on the fifth day. The 10 babies on routine feedings had an average level of 5.41 milligrams per cent on the fifth day; the 11 on reduced fat intake had an average level of 4.21 milligrams per cent; the 9 on a practically fat-free diet had an average level of 3.09 milligrams per cent.

TABLE 1. SERUM BILIRUBIN LEVELS OF HUMAN INFANTS FED DIETS CONTAINING VARYING AMOUNTS OF FAT

REGULAR FORMULA 3.6% FAT			REDUCED FAT 1.8% FAT			MINIMAL FAT 0.03% FAT			HIGH FAT 5.5% FAT[1]		
No.	Cord blood	5-day blood	No.	Cord blood	5-day blood	No.	Cord blood	5-day blood	No.	Cord blood	5-day blood
1	2.290	[2]	1	2.217	1.430	1	1.668	9.552	1	0.955	0.966
2	1.260	0.741	2	1.082	8.882	2	2.259	4.440	2	0.325	4.340
3	2.160	10.565	3	0.142	8.946	3	1.301	0.904	3	1.902	1.350
4	1.607	[2]	4	0.399	0.436	4	0.974	3.247	4	0.469	0.325
5	1.607	1.516	5	0.473	3.203	5	1.035	3.141	5	0.732	0.489
6	0.954	[2]	6	1.001	7.715	6	0.955	0.825	6	[2]	14.140
7	2.034	[2]	7	1.441	2.722	7	1.302	2.140	7	1.260	2.980
8	0.826	5.130	8	0.399	2.365	8	1.402	1.360	8	1.260	2.110
9	0.900	0.366	9	2.123	3.820	9	5.220	[2]	9	0.309	0.988
10	1.302	[2]	10	1.572	1.664	10	0.980	2.230	10	0.130	4.060
11	1.481	[2]	11	0.900	1.112						
12	1.789	[2]	12	3.495	8.250						
13	1.068	[2]									
14	0.751	2.350									
15	1.145	[2]									
16	2.351	11.354									
17	0.863	6.900									
18	1.088	1.989									
19	2.012	13.184									
Averages..	1.447	5.410		1.270	4.212		1.709	3.093		0.816	[1]

[1] Indicates that the diet was inadequately consumed and the figures can not be considered representative of the true status on this diet.

[2] Indicates no sample.

DISCUSSION

The explanation offered that the icterus of the newborn results because there is no need for so many oxygen-carrying units when the infant leaves the oxygen-poor environment of the uterus and enters the oxygen-rich outer world where its own lungs can afford better oxygen saturation is obviously teleological. That these elements are present seems to be apparent, but to afford the red cell with the ability to determine the end of its useful span is contrary to good scientific thought. The evidences of hemolysis are numerous. That the elevated serum bilirubin does not result primarily from tardy excretion of breakdown products of red blood cell destruction is clear (3, 4). Thus, the remaining factor of hemolysis must play a major rôle. Josephs *et al.* (14–16) clearly demonstrated the effect of fat ingestion on urobilin excretion in young infants. The rôle of fat in normal and abnormal red blood cell destruction has been reviewed by Johnson, Freeman and Longini (11) and has been shown to play a significant part in the breakdown of cells. Further support for the hemolytic thesis is gathered from the fact that the fragility of the erythrocyte in the newborn is normal at birth and becomes gradually increased in several days (4).

The findings in the present experiments do not provide a complete answer to the problem. It would appear that approximately 50 per cent of the destruction could be ascribed to the presence of circulating free fatty acids in the blood. However, numerous features are unanswered by these experiments. No effort was made to determine the amounts of bilirubin excreted in the urine and feces. Also, the total quantity of fat ingested was not closely regulated. That these factors might appreciably alter the findings seems likely, but whatever the absolute part of the hyperbilirubinemia might be which results from the hemolytic action of the circulating free fatty acids liberated from the ingested fats, these findings clearly indicate hemolysis of a significant degree.

SUMMARY

1. Infants are born with serum bilirubin levels of approximately 1.36 milligrams per cent.

2. On the fifth day of life, infants on routine feedings had a serum bilirubin level of 5.41 milligrams per cent, those fed on reduced fat formulas had a level of 4.21 milligrams per cent, and those fed on practically fat-free diets had a level of 3.09 milligrams per cent.

3. These findings indicate that approximately 50 per cent of the hyperbilirubinemia of the newborn can be explained on the basis of hemolysis resulting from the ingestion of fat.

REFERENCES

1. Elvehjem, C. A., W. H. Peterson and D. R. Mendenhall. Am. J. Diseases Children **46**: 105, 1933.
2. Musgrave, E. R. and M. I. Andresen. Am. J. Diseases Children **51**: 775, 1936.
3. Waugh, T. R., F. T. Merchant and G. B. Maughan. Am. J. Med. Sci. **198**: 646, 1939.
4. Waugh, T. R., F. T. Merchant and G. B. Maughan. Am. J. Med. Sci. **199**: 9, 1940.
5. Davidson, L. T., K. K. Merritt and A. A. Weech. Am. J. Diseases Children **61**: 958, 1941.
6. Johnson, V. and L. W. Freeman. This Journal **124**: 466, 1938.
7. Freeman, L. W. and V. Johnson. This Journal **130**: 723, 1940.
8. Loewy, A., L. W. Freeman, A. Marchello and V. Johnson. This Journal **138**: 230, 1943.

9. Freeman, L. W., A. Loewy and V. Johnson. This Journal **140:** 556, 1944.
10. Johnson, V., J. Longini and L. W. Freeman. Science **97:** 400, 1943.
11. Johnson, V., L. W. Freeman, and J. Longini. J. Am. Med. Assoc. **124:** 1250, 1944.
12. Evelyn, K. A. J. Biol. Chem. **115:** 63, 1936.
13. Malloy, H. T. and K. A. Evelyn. J. Biol. Chem. **119:** 481, 1937.
14. Josephs, H. W., L. E. Holt, H. C. Tidwell and C. Kajdi. J. Clin. Invest. **17:** 532, 1938.
15. Josephs, H. W. Bull. Johns Hopkins Hosp. **55:** 154, 1934.
16. Josephs, H. W., L. E. Holt, H. C. Tidwell and C. Kajdi. Bull. Johns Hopkins Hosp. **71:** 84, 1942.
17. Johnson, V., L. W. Freeman, and J. Longini. Ann. Rev. Physiol. **5:** 1945.

PRODUCTION OF KETOSIS BY THE GROWTH AND ADRENOCORTICOTROPIC HORMONES[1]

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The production of increased ketonuria in normal rats by injection of crude anterior pituitary extracts was first reported by Burn and Ling in 1930 (1) and subsequently Magistris (2) and Anselmino and Hoffmann (3) showed that there was an associated increased ketonemia. Insofar as we are aware, the production of ketosis by pituitary hormones which are both biologically and chemically pure has not previously been reported.

METHODS

In these experiments urinary ketone bodies were determined by the method of Van Slyke with Denige's reagent (4), blood ketone bodies by the method of Weichselbaum and Somogyi (5) and nitrogen by the micro-Kjeldahl procedure. All animals used were male rats of the Long-Evans strain and, except as noted in table 1, were between 80 and 100 days of age at the time of the experiment. Previous to the experiment they were maintained on the stock diet consisting of ground whole wheat 68½ per cent, fish oil 5 per cent, casein 5 per cent, alfalfa leaf meal 10 per cent, fish meal 10 per cent and sodium chloride 1½ per cent. The crude extract used was prepared according to the method of Evans *et al.* (6). The growth and adrenocorticotropic hormones were prepared according to the previously published methods of Li, Evans and Simpson (7, 8).

When the degree of ketonuria was to be determined, the urine was collected under toluene for the last 48 hours of a 72-hour fast, and the hormone was administered only during the fasting period. Growth hormone and crude extract were administered twice daily intraperitoneally while adrenocorticotropic hormone was administered three times a day via the same route. When blood ketone bodies were studied the animals were fasted 20 hours, a 0.3 ml. sample of tail blood was obtained with the animals under evipal anesthesia, and the first hormone injection was made. The hormone was then injected at hourly intervals and the second 0.3 ml. of tail blood was obtained with the animals again under evipal anesthesia three hours after the initial injection of hormone. In some instances immediately after obtaining the second blood sample, a functional evisceration was done according to the method of Selye and Dosne (9); the animals were then nephrectomized and a third blood specimen obtained from the inferior vena cava 30 minutes after completion of this procedure. These operative procedures required only about two minutes.

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[1] Aided by grants from the Research Board of the University of California, and the Rockefeller Foundation of New York City.

RESULTS

In table 1 are presented the results of the experiments in which ketonuria was determined. It will be seen that only the largest dose of crude extract (54 mgm.) produced a significant increased ketonuria while the same amount of boiled extract was without effect. Both growth and adrenocorticotropic hormones at a dose of 2 mgm. produced a significant and more marked ketonuria than did the crude extract. With the crude extract and with both of the pure hormones the excretion of β-hydroxybutyric acid increased to a greater extent than did the excretion of acetone and acetoacetic acid. In the controls the ratio of acetone and acetoacetic acid to β-hydroxybutyric acid was 0.44 while in the experimental animals it was 0.17, 0.28, and 0.26 for the crude extract, the growth hormone and the adrenocorticotropic hormone, respectively.

TABLE 1. URINARY KETONES AND NITROGEN EXCRETED BY FASTING MALE RATS DURING THE LAST 48 HOURS OF A 72-HOUR FAST

TOTAL DOSE OF HORMONE ADMINISTERED	TOTAL KETONES PER 100 GRAMS BODY WEIGHT		TOTAL NITROGEN PER 100 GRAMS BODY WEIGHT	
	mgm.	*p*[4]	*mgm.*	*p*
Controls[1] no injection	5.0 ± .63[3] (51)[2]		100 ± 3.1 (45)	
54 mgm. crude extract	12.5 ±2.7 (20)	<.01	83 ±16 (15)	<.01
27 mgm. crude extract	7.1 ±1.9 (11)	.20	77 ± 8 (11)	<.01
13½ mgm. crude extract	5.0 ±1.5 (12)	1.00	98 ± 4 (12)	.70
54 mgm. boiled crude extract	5.8 ±1.9 (9)	.50	105 ±14 (9)	.50
Growth hormone 2.0–2.5 mgm.	21.4 ±5.0 (14)	<.01	87 ± 3 (14)	.04
Adrenocorticotropic hormone 2.0 mgm.	20.9 ±5.3 (10)	<.01	107 ±10 (10)	.40

[1] Including 13 rats between 50 and 60 days of age.

[2] The figure in parentheses is the number of observations in the group.

[3] The standard deviation of the mean.

[4] From Fisher's table of *t*. A value of .01 or less is highly significant.

The nitrogen excretion of individual animals in the control group showed a significant negative correlation when compared with the ketone excretion, the correlation coefficient being −0.402 with a *p* value of 0.015. This negative correlation was lost when ketosis was produced by crude extract, the correlation coefficient being −0.044. This observation is perhaps due to the fact that the crude extract contained growth and adrenocorticotropic hormone both of which are ketogenic but which have opposing effects upon nitrogen excretion in the fed animal (13), the former decreasing and the latter enhancing nitrogen excretion. The effect of these hormones upon the nitrogen excretion of the fasting rat is not as striking as their effect upon the nitrogen excretion in the fed rat but the same trend is present, as shown by the data in table 1.

In table 2 are presented the results of the experiments in which blood ketone bodies were determined. It will be seen that neither the control animals nor the lactogenic hormone-treated animals showed an appreciable change in the level of blood ketone bodies comparing the initial and the three-hour specimens.

Both growth hormone and adrenocorticotropic hormone produced a significant rise in total blood ketone bodies in three hours. This not only confirms the ketogenic effects of these hormones but also demonstrates that the increased ketonuria could not have been due solely to a reduction in the renal threshold. The negative results with the lactogenic hormone demonstrates that the effect was a specific one and was not due to a non-specific protein effect.

Since the production of ketosis could be due either to an increased rate of formation of ketone bodies or a decreased rate of their utilization and since it is generally conceded that ketone bodies are formed almost exclusively by the liver and that they are utilized by non-hepatic tissues, the experiments using eviscerated-nephrectomized preparations were undertaken in an effort to determine whether over-production or under-utilization was the basic mechanism producing the observed ketosis. This procedure excluded both hepatic formation and renal excretion and the subsequent change in blood level was presumptively a measure of the rates of utilization of ketone bodies by tissues other than the liver, the gastro-intestinal tract and the kidneys. The results of these experiments are presented in table 3. It will be seen that the disappearance of blood ketone bodies subsequent to evisceration and nephrectomy was not significantly different in the experimental and control groups. This leads to

TABLE 2. Effect of growth and of adrenocorticotropic hormones[1] on the total blood ketone bodies of 20-hour fasted male rats

HORMONE USED	INITIAL BLOOD LEVEL		BLOOD LEVEL 3 HOURS AFTER THE FIRST INJECTION		CHANGE	
	mgm. %	*p*	*mgm. %*	*p*		*p*
Controls (12) no injection	26.4 ±2.7		24.9 ±2.8		−1.5 ±1.6	
Adrenocorticotropic hormone (8)	31.5 ±5.9	.40	47.5 ±6.7	<.01	+16.0 ±3.6	<.01
Growth hormone (8)	25.6 ±2.9	.80	37.8 ±2.6	<.01	+12.2 ±3.1	<.01
Lactogenic hormone (8)	24.1 ±2.4	.60	23.9 ±2.9	.80	− 0.2 ±1.0	.60

[1] A total dose of 2 mgm. was used in each case except for one rat in the ACTH-treated group that received 3 mgm.

TABLE 3. Decrease of blood ketone bodies in rats after evisceration and nephrectomy

	BLOOD LEVEL AT TIME OF EVISCERATION	LEVEL 30 MINUTES AFTER EVISCERATION AND NEPHRECTOMY	CHANGE	
				p
	mgm. %	*mgm. %*		
Controls (7)	29.9 ±3.9	7.6 ±1.4	−22.3 ±3.1	
Adrenocorticotropic (8)	45.5 ±7.5	10.8 ±2.2	−34.6 ±6.3	.15
Growth (5)	35.8 ±1.4	14.8 ±2.0	−21.0 ±2.4	.70

the conclusion that the ketosis was due to over-production rather than to underutilization. The rate at which blood ketone bodies disappeared was actually a function of the blood level at the time of evisceration, being most rapid in those animals with the highest blood level. A scatter diagram of the individual data which demonstrate this fact is presented in figure 1.

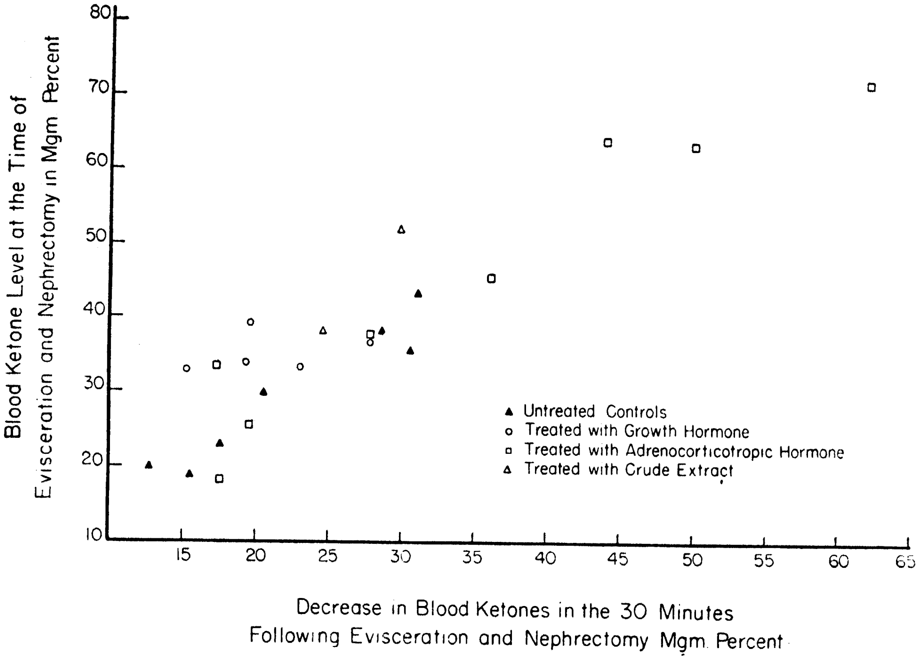

FIG. 1

TABLE 4 EFFECT OF GROWTH AND OF ADRENOCORTICOTROPIC HORMONES ON THE TOTAL BLOOD KETONE BODIES OF ADRENALECTOMIZED RATS

	INITIAL BLOOD LEVEL		BLOOD LEVEL 3 HRS. AFTER THE FIRST INJECTION		CHANGE	
	mgm. %	*p*	*mgm. %*	*p*		*p*
Controls (6)	35.8 ±5.7		25.9 ±6.4		−9.9 ±1.4	
Growth hormone (6)	29.4 ±2.7	.25	33.3 ±4.2	.30	+3.9 ±2.4	<.01
Adrenocorticotropic hormone (5)	28.8 ±3.4	.30	26.3 ±7.6	.95	−2.5 ±5.2	.12

In an effort to determine whether the adrenal cortex participated in the production of ketosis by growth and adrenocorticotropic hormone, a fourth experiment was undertaken in which the effect of these hormones on total blood ketone bodies of adrenalectomized animals was studied. These animals were adrenalectomized 3–5 days prior to the experiment, were then maintained on a one per cent sodium chloride solution as drinking water, and were fasted only

16 hours before taking the first blood sample. The data from this experiment are presented in table 4. It will be seen that under these conditions both the control animals and the adrenocorticotropic hormone-treated animals showed a decrease in blood ketones in the three-hour experimental period. The growth hormone produced an increase in the blood level which was significant when compared with the change in the control group. Although we do not feel that these results are conclusive, they suggest that the ketogenic effect of growth hormone is independent of the adrenal cortex while the ketogenic effect of adrenocorticotropic hormone is not.

DISCUSSION

While it is not within the scope of this paper to present an exhaustive review of the literature it may be pointed out that these results have bearing upon some previously reported work. For example, Greaves (10) has reported that growth-promoting and ketogenic activities were associated during the fractionation of crude extract, and Shipley and Seymour (11) have reported the failure of ultrafiltration to separate growth and ketogenic effects. These results may be explained by the ketogenic effect of the growth hormone itself. The fact that adrenalectomy reduces the ketogenic effects of partially purified pituitary extract (12) may be due to the fact that the adrenocorticotropic component of the extract is no longer effective.

With respect to the mechanisms involved in the production of ketosis by these two hormones one can only speculate. Since the growth hormone reduces nitrogen excretion, produces protein storage and decreases body fat, under the conditions of these experiments there must have been a relative increase in fat oxidation which could have resulted in the observed ketogenic effect. Adrenocorticotropic hormone increases endogenous protein catabolism and since some amino acids are precursors of ketone bodies this may in part account for the ketogenic effect found. In addition, adrenal cortical steroids inhibit carbohydrate oxidation and the increased production of cortical steroids under the influence of adrenocorticotropic hormone may have resulted in a relative increase in fat oxidation.

The production of ketosis by two chemically pure hypophyseal hormones invalidates the concept that there is a single ketogenic factor in the anterior lobe. It is entirely possible that other hypophyseal hormones possessed of metabolic activity may also produce ketosis as one of their manifestations.

SUMMARY

1. Pure growth and pure adrenocorticotropic hormone increased the ketonemia and the ketonuria of fasted normal rats.

2. The ketogenic activity of both hormones was due to an increase in the rate of production rather than due to a decrease in the rate of utilization of ketone bodies.

3. Pure lactogenic hormone was without effect upon blood ketone bodies.

BIBLIOGRAPHY

1. Burn, J. H. and H. W. Ling. J. Physiol. **69**: XIX, 1930.
2. Magistris, H. Endokrinologie **11**:176, 1932.
3. Anselmino, K. J. and F. Hoffmann. Klin. Wochschr. **10**: 2380, 1931.
4. Peters, J. P. and D. D. Van Slyke. Quantitative Clinical Chemistry, 1st ed. Baltimore: Williams & Wilkins Co., 1932.
5. Weichselbaum, T. E. and M. Somogyi. J. Biol. Chem. **140**: 5, 1941.
6. Evans, H. M., K. Meyer and M. E. Simpson. Mem. Univ. Calif. Vol. II, 1933.
7. Li, C. H., H. M. Evans, and M. E. Simpson. J. Biol. Chem. **159**: 353, 1945.
8. Li, C. H., H. M. Evans, and M. E. Simpson. J. Biol. Chem. **149**: 413. 1943.
9. Selye, H. and C. Dosne. This Journal **128**:729, 1940.
10. Greaves, J. D., I. K. Freiberg and H. E. Johns. J. Biol. Chem. **133**: 243, 1940.
11. Shipley, R. A. and W. B. Seymour. Endocrinology. **31**: 634, 1942.
12. Shipley, R. A. Endocrinology **26**: 900, 1940.
13. Gordan, G. S., C. H. Li and L. L. Bennett. Proc. Soc. Exp. Biol. and Med. **62**: 103, 1946.

EFFECT OF INORGANIC PHOSPHATE ON THE GLYCOLYSIS OF HUMAN BLOOD[1]

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In the paper by Hald, Heinsen and Peters (1) it was reported that inorganic phosphate was transferred across the red cell membrane when an isotonic solution of phosphate buffer was added to defibrinated human blood. Experiments are presented in this note to demonstrate that this transfer is associated with a change in the metabolism of the cells and is not due merely to the high concentration gradient of phosphorus.

Roche and Roche (2) showed an increased rate of glycolysis when phosphate was added to blood. This was first verified, after which transfers of phosphate were compared with concentrations of glucose.

PROCEDURE AND RESULTS

Glucose was measured by the Benedict method (3) on blood filtrates prepared according to Somogyi (4). All other technical procedures and methods of calculation were carried out as before (1).

After blood had been fortified with glucose, 6 cc. aliquots were oscillated at 37°C. for 20 or 21 hours with and without additions of one cc. of isotonic solutions of sodium chloride or phosphate. The latter were composed of sodium or potassium salts in proportions of dibasic : monobasic phosphate = 4:1. The concentrations of glucose in the original blood and in each incubated sample were determined. The results are shown in table 1. When glucose solutions of 550 and 150 mgm. per cent were incubated with the isotonic solutions in the same manner, the concentration of glucose did not fall.

Isotonic solutions of sodium chloride and of phosphate buffers were then added to twice their volume of blood, and the diluted blood samples were equilibrated at 37°C. with 40 mm. of CO_2 in air. The total time which elapsed between sampling the original blood and the equilibrated bloods was less than one hour. The original and derived data are shown in table 2. Addition of the isotonic solutions to twice their volume of a 200 mgm. per cent glucose solution gave theoretical recoveries of glucose, proving that the buffer solutions did not have any effect upon the Benedict glucose method.

The rate of glycolysis of incubated blood was increased by the addition of phosphate. With equilibrated blood, the controls with saline showed a slight variable fall in glucose. The blood with added phosphate showed the transfer

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[1] Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

TABLE 1. EFFECT OF INCUBATING ISOTONIC SODIUM CHLORIDE AND PHOSPHATE BUFFER WITH BLOOD

EXPERIMENT	ORIGINAL GLUCOSE	INCUBATION TIME	FALL OF GLUCOSE		
			Mgm. per 100 cc. original blood per hour		
			No addition	NaCl	Phosphate
	mgm. %	*hrs.*			
1	488	20	8.9	9.1	13.0[1]
2	590	21	14.0	13.0	15.5[1]
3	632	20	13.8	12.8	16.1[2]
4	526	20	13.8	13.0	16.2[2]

[1] Sodium phosphate. [2] Potassium phosphate.

TABLE 2. THE EFFECTS OF ADDING ISOTONIC PHOSPHATE BUFFER TO BLOOD

EXPERIMENT	ORIGINAL DATA					DERIVED DATA			
			Phosphorus			Total amount of			
	Cell vol.	Blood glucose	Inorganic		Organic	Blood glucose fall	Phosphorus		
							Inorganic		Organic
			Blood	Serum	Blood		Serum	Cells	Cells
	%	*mgm.%*	*mM./l*	*mM./l.*	*mM/l.*	*mgm.*	*mM.*	*mM.*	*mM.*
1. No addition	50.0	109	0.92	1.05	7.40	x	0.52	0.40	7.4
Na-phosphate	33.3	63	35.7	45.0	4.0	x + 14	−6.3	8.6	6.0
Change						14	−6.8	+8.2	−1.4
2. Original		296							
NaCl	26.9	188	0.65	0.62	3.94	14	0.67	0.29	5.9
Na-phosphate	26.9	174	36.0	43.3	2.8	35	−3.8	6.5	4.2
Change						21	−4.5	+6.2	−1.7
3. Original		306							
NaCl	33.1	195	0.76	0.77	4.46	13	0.78	0.37	6.7
Na-phosphate	33.1	181	36.4	45.0	3.0	34	−6.1	9.5	4.5
Change						21	−6.9	+9.1	−2.2
4. Original		253							
NaCl	34.9	168	0.68	0.58	4.80	1	0.57	0.45	7.2
K-phosphate	34.9	152	36.5	45.8	3.7	25	−7.4	10.1	5.5
Change						24	−8.0	+9.7	−1.7
5. Original		210							
NaCl	30.1	125	0.75	0.75	4.30	22	0.79	0.33	6.5
K-phosphate	30.1	120	36.9	44.8	2.9	30	−5.2	8.4	4.4
Change						8	−6.0	+8.1	−2.1

of inorganic phosphate noticed previously, which was accompanied by a consistent acceleration of glycolysis.

SUMMARY

The addition of isotonic phosphate buffers to human blood causes inorganic phosphate to enter the red blood cells. This transfer is associated with an increased rate of glycolysis.

REFERENCES

1. Hald, P. M., A. J. Heinsen and J. P. Peters. This Journal **152:** 77, 1948.
2. Roche, A. and J. Roche. Bull. Soc. Chim. Biol. **11:** 549, 1929.
3. Benedict, S. R. J. Biol. Chem. **68:** 759, 1926.
4. Somogyi, M. J. Biol. Chem. **86:** 655, 1930.

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NEURAL RESPONSES AND REACTIONS OF THE HEART OF A HUMAN EMBRYO[1]

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The observations recorded in this paper were made on the isolated heart of a human embryo of 100 millimeter crown-rump length. Such a size on the basis of age-length curves (1) would indicate a presumptive fertilization age of 13 weeks. The isolated ventricles of the heart, after removal in preparation for experiments, were nine millimeters from base to apex.

The immediate object of the study was to determine whether cholinergic inhibitory nerves (vagi) would function at this embryonic stage and to compare other functional characteristics with those accredited to adult mammalian hearts.

The observations followed a procedure which has been used to advantage by the authors in unpublished studies on the hearts of several mammalian forms. It consists simply of initial rapid chilling of the heart, in a modified Ringer's solution, to about 5°C. and the subsequent study of cardiac strips at temperatures lower than the normal body temperature. In such a cold solution mammalian cardiac tissue will remain quiescent but viable often as long as four days; strips of either atrium or ventricle will beat spontaneously when the temperature is raised, and at room temperature (e.g., 24°C.) the beats are more vigorous than at body temperature. When adequately oxygenated the beats will continue for hours, and such strips have been used on three successive days after cooling to 5°C. during the night.

The embryonic heart was divided into three preparations, the sinus-atrium and two ventricular portions, the dissection being completed in the cold Ringer's solution. Records of contractions were made by the simplest suspension

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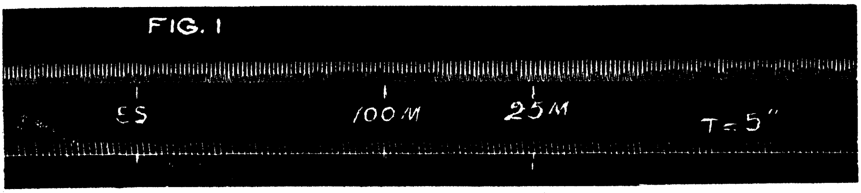
[1] This investigation was aided by a grant from the American Medical Association.

method. For recording, the preparation was attached to a light magnesium ribbon lever and immersed in 50 cc. of saline solution, through which oxygen was gently bubbled. The buffers of the solution maintained a pH of 7.5 to 7.6 throughout all observations. The test for vagal inhibition was that described for cold-blooded hearts by Garrey and Chastain (2). After eserinizing, acetylcholine was added to a desired concentration to produce stimulation of the inhibitory nerves. The inhibitory action of the vagi on the pacemaker and atrial contraction of mammalian hearts is strikingly effective by this method of testing, and effects are obtained at low temperatures as well as at body temperature, hence its use in the present investigation.

Ventricles. The right ventricular muscle was mounted for recording and gradually warmed from 5°C. to room temperature (24°C.). After a half hour the preparation contracted in response to electrical stimulation and an occasional automatic beat was observed. These contractions were not weakened by eserine (1–20,000) plus acetylcholine bromide in concentrations up to one part in 10,000 of saline. After repeated washing with Ringer's solution, the ventricular muscle was subjected to a continuous bath of one in one million acetylcholine bromide. In this solution it responded to induction shocks and a regular rhythm developed. These spontaneous beats appeared at first in groups which changed in such a way as to indicate the disappearance of partial block between the point of impulse initiation and the main muscular mass. Once established the beats continued with force and regularity at a rate of 17 per minute at 24°C. Thus the presence of acetylcholine did not, through any inhibitory action, prevent the development or conduction of autogenous contractions. Two hours later the heart was beating strongly; then, following further treatment with eserine (1–50,000), the concentration of acetylcholine was raised to one in 100,000 and finally to one in 25,000 without any evidence of inhibition of rate or force of contractions (fig. 1).

Adrenaline chloride (one in 250,000) failed to elicit significant effects beyond a slight acceleration of rate. It was not determined whether this was due directly to a muscular action or indirectly to the influence of functionally active sympathetic nerves. Identical reactions were obtained from our second ventricular preparation, the contractions of which were at the identical rate of the first preparation, a rate less than one-third that of the sinus-auricular preparation. It would thus appear that the rhythmogenic characteristics and ratios which are found in different parts of the heart of the adult are already established prior to the age of this embryo. Histological preparations revealed the presence of many nerve fibers throughout the ventricles; nevertheless, there was a complete absence of any cholinergic inhibitory effects. This, however, is not surprising since, by use of this same method of testing, we have been unable to demonstrate cholinergic inhibition of the adult ventricular muscle of any of the many types of mammals studied.

Sinus-atrium. Three hours after its initial cooling the auricular preparation was mounted for recording at room temperature. The beats gradually increased to a uniform height and continued at a rate of about 63 per minute. Unlike the ventricles this atrial preparation did show definite, though slight, evidences of inhibitory responses when the acetylcholine tests of vagal inhibition were applied, but the sensitivity was low. For example, after anticholinesterase treatment with eserine (one in 50,000) this preparation did not react in any detectable way to acetylcholine bromide unless the concentration was greater than one part in 10 million, which caused a 20 per cent reduction in the height of contraction followed by a partial block between the sinus (pacemaker) and the atrium, resulting in a sudden drop of atrial contractions to one-half the pre-existing rate. The latent period of the drug action was very long, for the partial block did not appear until nearly two minutes after application of the acetylcholine. At this juncture the concentration of acetylcholine was quadrupled (one in 2.5 million). Again after a latent period of nearly two minutes the partial block suddenly became complete and the atria remained quiescent. Inhibition, however, was far from complete since they responded with contractions upon electrical stimulation. Release from the effects of acetylcholine followed 45 seconds after treatment with atropine (one in one million); the block disappeared, the rate accelerated



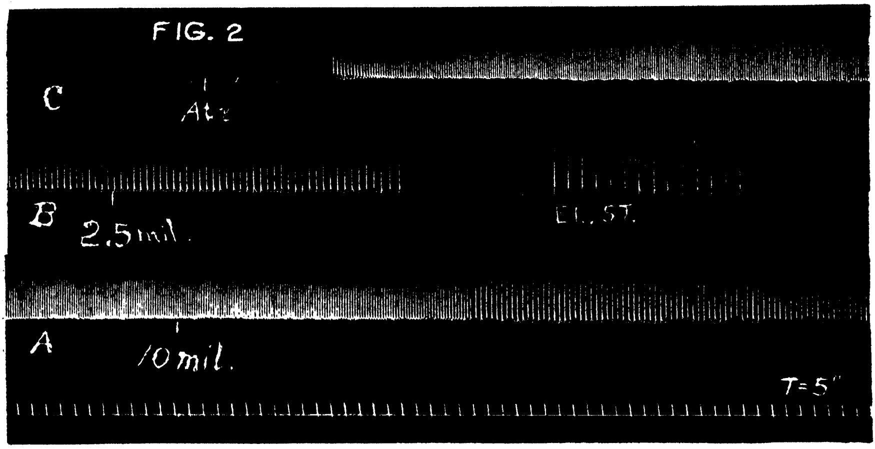


FIG. 1. Automatic beats of the ventricle. Not inhibited by eserine (ES, 1/50000) plus acetylcholine one in 100,000 (at 100 M) or one in 25,000 (at 25 M). Time tracing = 5″ intervals.

FIG. 2. Sino-atrial beats. *A*, *B* and *C* are successive records. *A* records the long latency and 2/1 block produced by acetylcholine (one in ten million, 10 mil.). *B* records the complete s/a block produced by stronger acetylcholine solution (one in 2.5 million, 2.5 mil.), but contractions were elicited by electrical stimuli. *C* shows the release from acetylcholine action when atropine, one in one million, was added at atr. The same time record (T = 5″) applies to all tracings.

promptly to the normal rate and the height of contractions which had been weakened to one fourth of their previous height progressively increased until they exceeded the norm by about 30 per cent (fig. 2).

Since atropine acts by paralyzing vagal action, these results are consistent with the interpretation that at this embryonic stage inhibitory function of the vagus is already demonstrable on the sinus rate (slight), on conduction and on the strength of atrial contraction, but the inhibition is slight indeed compared with that which the authors have obtained by the same method when applied to adult atria of several mammalian forms. In the adult heart inhibition results from extreme dilutions of acetylcholine, e.g., one part in 300 million. There is practically no latency and the weakening of the contractions is profound. Nerve cells and fibers were abundant in the atrial preparation of our embryo, yet the cholinergic inhibitory responses were feeble and their character and course was such as to suggest the possibility that at this early embryonic stage the inhibitory nerves function weakly and that their full functional potentialities are acquired only at a later stage of development. Such a delay in the development of its inhibitory action by the vagus of the chick has been reported by Pickering (3), and Barcroft found that vagal inhibition of the heart of the foetal lamb could be demonstrated only after the 88th day of gestation (cf. Barclay, Franklin and Prichard, 4).

In tests made for us by Dr. Charles E. King it was found that the intestinal muscles of this embryo responded promptly and vigorously to acetylcholine. The functional responses of the gastrointestinal tract were developed at this stage far in excess of any demonstrable in our cardiac tests.

Temperature reactions. The incidental observations on the effects of temperature referred to above were extended to include the entire gamut from 3° C. to 40° C., thus adding a record of the reactions of a human embryo of a specific age to the voluminous literature in this general field of temperature effects.

The sino-atrial preparation beat most vigorously at a temperature between 25° and 30°C. When cooled to 20° and then to below 15°, the preparation maintained vigorous beats at progressively slower rates. At 10° the atrial contractions suddenly ceased, although effective responses were elicited by electrical stimuli even at 3°C. These results suggest that temperatures below 10° acted primarily by blocking sino-atrial conduction, an interpretation which was further supported by the fact that upon warming the preparation to 12° the atrial contractions reappeared suddenly with full vigor and with complete regularity, as if block had suddenly ceased. Such a result calls for tests for impulse formation in the sinus while the atrium is at rest below 10°C., tests which were not feasible during our experiments.

A graph in which rate of beat is plotted against temperature is shown in figure 3. It shows the slow rate at low temperature, deviating little from a straight line between 20° and 35°. Below 20° there is a definite deflection, giving the lower part of the well-known S-shaped curve so characteristic of the action of temperature on rhythmic processes. Temperature coefficient, calculated from the data of the graph, was greater than five for the ten degrees change in rate between 10° and 20°, while between 30° and 40° it was only 1.6. For the rectilinear portion of the graph the rate varied about seven beats for each centigrade degree.

At the normal body temperature of 37°C. this isolated embryonic heart beat at the rate of 157 per minute, which may be considered the fundamental pacemaker rate of the preparation. It is not without interest to note that the average mid-foetal heart rate is given as 156 per minute (Windle, 5) and that this intrinsic rate progressively slows to the time of birth, due presumably to the development of vagal tone during the later stages of foetal life and to its inhibitory impress which maintains the slower rate throughout life. With removal of vagal influences either by section of the nerves or by atropinization, as is well known, the release from tonic inhibition reestablishes the rapid rate which approximates the fundamental rhythm of the embryonic heart prior to the development of vagal inhibitory tone.

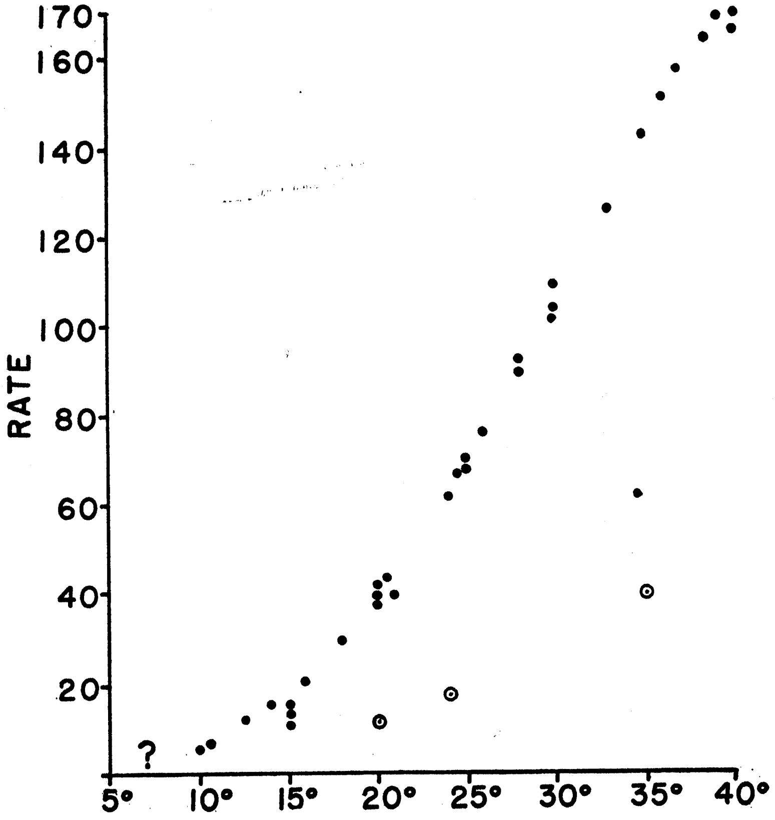


FIG. 3. Heart rates (*ordinates*) are plotted against temperatures (*abscissae*). Solid dots represent rates of the sinus-atrium. The three circles indicate ventricular rates.

In the case of the beating ventricular preparation at 24° the rate was 17 per minute as compared with a sino-atrial rate of 62, giving a ratio of one to 3.6. Observations were made at only two other temperatures; at 20° the ventricular rate was 12 while that of the atrium was 40 per minute and at 35° the ventricular rate was 39 per minute while that of the atrium was 142 per minute. The ratios in the two instances were 3.3 and 3.6, respectively. It would appear from these figures that the rhythmogenic characteristics of sino-atrium and ventricle include an intrinsic rate ratio not far from 3.5/1, that this relationship is established prior to the age of our embryo (13 weeks) and corresponds in general to the status known to exist in adult hearts.

SUMMARY

1. Isolated and previously chilled preparations of the atrium and of the ventricles of a 13-weeks human embryo beat for hours at room tempeature in well oxygenated Ringer's solution.

2. Acetylcholine even in high concentration had no inhibiting effects on the ventricles.

3. There was some inhibitory action of acetylcholine on the sinus-atrium. The effects were produced only by strong solutions and were delayed in their onset even one or two minutes. Profound inhibition, such as the authors have seen with other postpartum mammalian hearts, did not occur.

4. The results suggest that the full inhibitory function of cardiac nerves is a later foetal development.

5. The isolated sino-atrial preparation beat at all temperatures between 10°C. and 40°. The intrinsic sinus rate at 37°C. was 157 per minute. The ratio of this rate to the slower idioventricular rate was about 3.5/1. These rates approximate those of the adult heart when free of nervous regulation and indicate that the rhythmicity 'pattern' of the heart is established early in embryonic life.

We are indebted to Dr. Mary E. Gray for the histological examination of this heart, prepared with Ranson's pyridine silver stain. We summarize her findings as follows: Many large nerve trunks following blood vessels in both auricles and ventricles. Many fine nerve branches among the muscle cells of the entire heart. Numerous ganglion cells in the subepicardial connective tissue of the auricles only. No ganglion cells were found in the ventricles.

REFERENCES

1. **Patten**, B. M. Human Embryology, Philadelphia: The Blakiston Company, 1946. P. 185.
2. **Garrey**, W. E. **and** L. L. **Chastain**. This Journal **119**: 314, 1937.
3. **Pickering**, J. W. J. Physiol. V. **20**: 165, 1896.
4. **Barclay**, A. E., K. J. **Franklin and** M. L. **Prichard**. The Foetal Circulation, Oxford: Blackwell Scientific Publications, Ltd., 1944. P. 112.
5. **Windle, William** F. Physiology of the Fetus. Philadelphia: W. B. Saunders and Company, 1940. P. 17 et seq.

EXPERIMENTAL HYPOTHERMIA AND REWARMING IN THE DOG RECOVERY AFTER SEVERE REDUCTION IN BODY TEMPERATURE[1]

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In studies of the hypothermic state, observations have been made upon the capacity for recovery following rather drastic reduction of body temperature, and certain points of interest are incorporated in the present report. It is noteworthy particularly in view of the degree of cooling which can be sustained under certain conditions with the sequel of a complete recovery.

METHODS

Dogs of an unselected but generally healthy stock were used. The procedure followed consisted simply in submerging the undepilated[3] and lightly anesthetized preparation to the neck in a bath of iced water; immersion was continued until a crisis, respiratory or cardiac, supervened. In some instances, following partial rewarming, animals were subjected to re-immersion. While recourse to anesthetics in this type of experiment is difficult of defense on a strictly physiological basis (1) it proved desirable from certain standpoints to induce an initial narcosis sufficient at least to offset the intense psychomotor disturbances otherwise incurred by abrupt immersion in a chilled bath. Moreover, light anesthesia afforded an initial facilitation of reduction in temperature which is otherwise very difficult to achieve (2). Accordingly, an anesthetic agent of short-acting nature was used—either pentothal sodium *per venam* in sedative dosage, pentothal supplemented with ether, or ether alone, withdrawn as soon as feasible. In the final experiments cyclopropane was employed, and proved most satisfactory of all. During immersion (bath temperatures ranging from 2° to 9°C) records were kept of pulse, respiration, deep rectal temperature (4–6 cm.) and, occasionally, deep visceral and muscle temperature. Periodic electrocardiograms were recorded.

The immersion period ranged from 67 to 193 minutes (avg. 155); sudden collapse of pulse or of respiration signalled the institution of rewarming measures. Artificial respiration was occasionally required. Rewarming was carried out routinely by immersion in water at 42° to 45°C.

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[1] This work was carried out under Contract W33-038 ac 14757 with the Aeromedical Laboratory, Air Materiel Command, A.A.F., Wright Field, Dayton, Ohio.

[2] Now in the Department of Pharmacology.

[3] *A series of trials failed to demonstrate an appreciable* influence of depilation upon rate *of cooling. This is in agreement with an observation by Spealman (2).*

RESULTS

Thirteen animals sustained reduction in rectal temperatures to an average of 14.9°C. (range 11.7° to 16.8°C.). These are listed in table 1. Recovery in each instance was complete, and the animals were returned to quarters within two hours of the immersion period, apparently little the worse for the experience. In two animals the rectal temperature was reduced to 15°C. or below on two occasions with an interlude of one week.

TABLE 1. Summary of cases surviving acute hypothermia

ANIMAL NO.	LOWEST RECTAL T°C.	REMARKS
1	16.4	Pulse 28, irregular; resp. 12, shallow. Hot water immersion; rapid recovery
2	15.0	Pulse and resp. ceased; artificial resp. and massage, resp. and pulse resumed in 5 min. Recovery. Shivering at 29°
3	15.2	No P wave in EKG. Hot water; shivering at 23.8; good recovery
4	13.0	Pulse 18, resp. 6, irregular. Rapid rewarming; shivering at 17°, pulse 72. Recovery
5	15.0	Pulse 18–20; irregular; rewarming; movements and whimpering at 24°. Rapid recovery.
6	16.0	Pulse 32, weak, irregular; artificial resp.; rewarming with immediate response; shivering at 20°
7	15.0	Pulse 2–3; resp. 4, shallow; rewarming, pulse rose to 20 before rectal t° responded; shivering at 26°
8	14.8	Pulse 24, irregular, resp. 6; rewarming; shivering at 15°. Rapid recovery.
9	15.0	Resp. 3, pulse impalpable; rewarming; at 24° voluntary movements. Good recovery.
10	14.2	Pulse 30, irregular; rewarming; shivering at 18.8; pulse 100. Recovery.
11	15.6	Pulse 32, resp. 8; rapid recovery on rewarming
12	11.7	Cyclopropane anesthesia; rewarming; knee jerk + rectal t° 17°
13	16.8	Pulse 16, irregular; spontaneous rewarming; rectal t° 20° in 120 minutes, pulse 58. Good recovery

Shivering appeared to exert no marked influence upon the rate of temperature fall, which was essentially linear (figs. 1, 2, 3). In the downward course, shivering began variously at rectal temperatures of 30° to 35.8°C.; during rewarming its onset occurred over a wide spread—ranging from 15°C. in one instance (Expt. 8) to 29°C. (Expt. 2). It varied widely in intensity and did not seem significantly to accelerate recovery.

Skeletal muscle tonus, greatly exaggerated during the cooling process, was not detectable below rectal temperatures of 20° to 18°C. and the intense rigidity characteristic of the cooling animal was succeeded by a flaccid state, in agreement with the observations of Crismon (3). In the rewarming process, tonus reappeared within the same temperature range, with recovery of superficial reflexes.

Eight animals failed to respond to warming, or succumbed before resuscitation measures were started. The cause of death in some cases appeared to be circulatory failure of cardiac origin. Associated with the marked degree of bradycardia which supervened as rectal temperatures fell below 20°C., engorgement of the heart and great veins was a consistent autopsy finding.

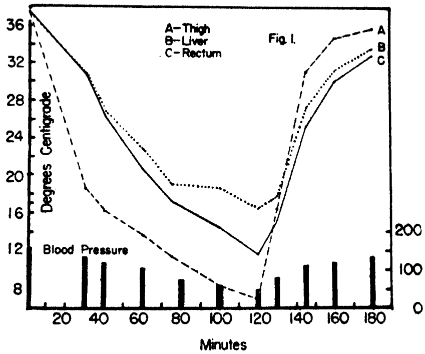

FIG. 1. EXPT. 12. 8.1 kgm. Cyclopropane anesthesia. Rewarming begun at 120 minutes. Automatic recording of temperature in this and other figures. Carotid blood pressure by photoelectrokymographic registration.

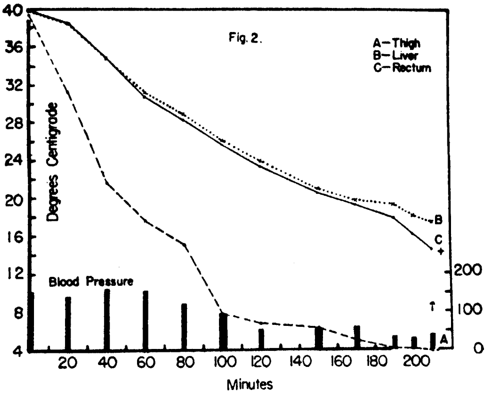

FIG. 2. EXPT. 18. 10.1 kgm. Pentothal sodium anesthesia. Removed from bath at ↑. Exitus at +.

The reliability of rectal readings as an absolute index of mean body temperature under conditions of immersion has, of course, been questioned (4) and it is to be expected that a deep-to-surface gradient exists. This is shown in the record of the hypothermia in one experiment which is incorporated in figure 1. The temperatures of three body regions (liver, rectum, thigh muscle) as obtained by thermocouple needles were recorded automatically[4]. It is seen, in general, that the liver retained the highest temperature during the cooling process with, in this case, a maximal liver-rectum gradient of 4.8°C. The graph illustrates as well the linear fall characteristic of mean blood pressure as the hypothermic state progresses. This is true as well of the pulse rate. In figure 2 is depicted the decline in temperatures and in blood pressure in an experiment in which, despite a slower rate of fall and a smaller liver-rectum gradient (maximum 2.9°C.), recovery was not effected; death supervened five minutes after the rewarming

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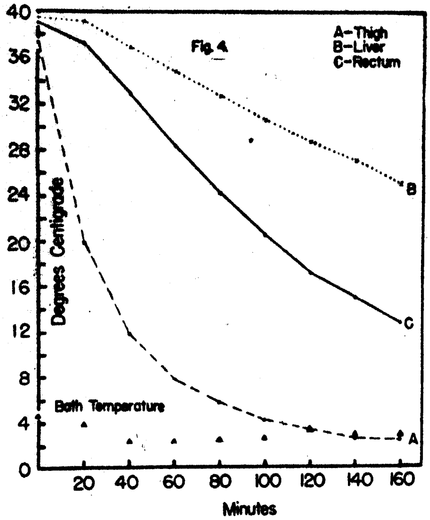
[4] 'Speedomax' recorder, Leeds & Northrup Company, Philadelphia.



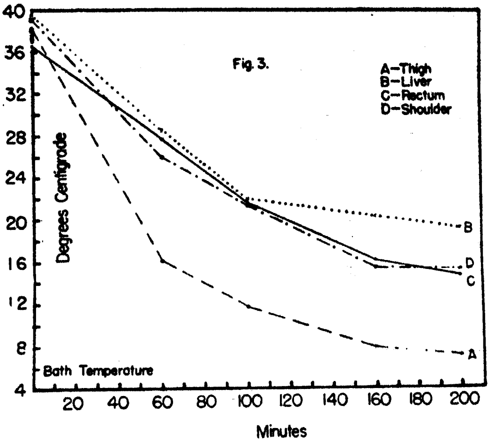


FIG. 3. AVERAGE OF THE REDUCTION IN TEMPERATURES in twelve experiments, for body regions as indicated. Average bath temperature 5.6°C.

FIG. 4. TEMPERATURE RECORDS in freshly sacrificed animal (11 kgm.) placed in iced water bath, showing marked lag in 'deep-to-surface' temperature gradient in the absence of circulation.

TABLE 2. DOG #14, FEMALE MONGREL, 5.5 KGM.; ETHER ANESTHESIA, ATROPINE 5 MGM. S. C.

TIME	RECTAL T°	REMARKS
0 min	38.8	Into bath at 4.5°
15 "	34.0	Slight shivering
35 "	26.0	Some muscular movements
55 "	19.0	Respiration 50
85 "	16.8	Pulse 22, irregular
110 "	15.0	Removed from bath
120 "	13.5	Respiration 14, pulse 18
135 "	12.5	Respiration 6
150 "	11.5	Rewarming begun: pulse 0., resp. 2
153 "	11.3	
164 "	14.5	Shivering begun
167 "	18.0	Respiration improving; knee jerk +
171 "	18.2	Respiratory gasps, followed by apnea, pulse feeble. Artificial respiration. No recovery

P.M. (173) min. Right auricle beating rhythmically; ventrical insensitive to irritation, enormously engorged. Lungs clear, viscera congested. No ascitic fluid.

was begun. The data of twelve experiments on comparative temperature readings are summarized in figure 3.

One clearly defined instance of 'rewarming death' was recorded, an abbreviated protocol for which is given in table 2. At a rectal temperature of 11.5°C. the pulse, noted a moment previously at eight per minute, temporarily ceased to register on the EKG recorder. Rewarming with artificial respiration was begun; a pulse reappeared, and in eleven minutes the rectal temperature had risen to 14.5°C., at which point shivering commenced. Seventeen minutes after rewarming was begun the rectal reading was 18°C., respiration 18, knee jerk positive. Twenty-one minutes after the start of resuscitation (rectal 18.2°C.) the animal began gasping, the pulse became feeble and erratic. Artificial respiration proved ineffective. This type of collapse has been noted (5, 6) and has been attributed to the overloading of the myocardium under conditions of hypoxia.

Electrocardiographic responses to progressive cooling will be discussed elsewhere. It is of interest to note, however, the remarkable slowing to which the cardiac cycle may be subjected. Cycles, for example, of 1.8 seconds have been recorded, the beats separated by extremely long diastolic pauses, with pulse rates as low as six per minute. Neither section of the vagi in the neck, nor atropinization appear to influence appreciably the course of the cardiac cycle under conditions of cooling. This is in accord with the findings of Grosse-Brockhoff and Schoedel (7).

DISCUSSION

Observations upon reversibility of hypothermic changes have been reported by a number of investigators. Britton (8), in one of the more comprehensive of earlier papers, remarked that cats, anesthetized with a chloroform-ether mixture and cooled in an air-chamber, 'may survive' cooling to a deep rectal temperature of 16°C.; recovery ocaurred more commonly if body temperature was not lowered below 20°C. Britton listed also the chronological reappearance of reflexes and reactions during the spontaneous rewarming process. More recently Crismon (3) and Spealman (2) have contributed pertinent observations. The former reported that application of warm water was rapidly effective in restoring the dog, provided either *a*) the circulatory and respiratory depression had not progressed beyond a certain point or *b*) the duration of hypothermia was restricted within rather definite limits. The tolerable hypothermia was not so severe in degree as that reported here. However, pentobarbital sodium was used as a pre-immersion anesthetic agent; in our experiment this barbiturate curtails the ability of the animal to withstand cold. In Crismon's experiments, for example, dogs were revived from rectal temperature levels below 24°C. only if permitted below this level for less than 20 minutes. In the present experiments animals have survived reduction to 14°, 15° and 16°C. rectal temperatures under circumstances in which the temperature was less than 24°C. for as much as 110 minutes (e.g., Expt. 10).

The use of substances such as pentobarbital sodium places the animal, in effect, in 'double jeopardy', for upon the depression induced by the initial anes thesia is superimposed the narcosis of hypothermia, and the organism has therefore the combined effects of the two to overcome. Pentothal sodium was used in our experiments on the assumption that as cooling progressed the effects were supplanted by, and not combined with, cold narcosis. Admittedly, the time required for the influence of pentothal to subside under cooling is conjectural; and estimates in this case may possibly be overly sanguine. However, animals administered cyclopropane behaved identically, and administration was required only until a temperature of 27° to 25°C. had been reached; beyond this point, cold narcosis sufficed and there is at present no reason to believe that the transitory depressant effects of cyclopropane, even under conditions of cold, are not rapidly dissipated with the blowing off of the agent.

Spealman utilized animals without benefit of anesthesia and found it difficult to obtain a marked hypothermia. Large dogs (averaging 27.6 kgm.), for example, did not cool appreciably in baths at 20°C. (68°F.) or above; at 15°C. (59°F.) and below, cooling could occur, but in some animals a normal temperature level was maintained for at least five hours in a bath at 0°C. 'Serious impairment' was shown when rectal temperatures did decline to about 27°C. The linear fall in rectal temperature, as reported by Crismon (3) and in the present report was due, in all probability, to the influence of the initial anesthesia in vitiating the capacity to combat increased heat loss (1). It seems a fair surmise that if Spealman's conscious animals could have been cooled to the stage of cold narcosis, the rate of cooling from that point on would have been a linear one. Indeed, the anesthetized animal differs but little from the dead animal so far as rectal cooling (from an initial 37°C. or above) in a cold bath is concerned; two experiments in which freshly sacrificed animals were immersed in iced water revealed a linear rate of heat loss comparable in point of time to that which characterized the living, but anesthetized, preparation. An understandable lag occurred in decline in visceral temperature, in view of the absence of circulation (fig. 4).

Species other than the dog have been examined as to viability under the stress of sharply reduced environmental temperatures, and species differences, as usual, have been revealed. Simpson and Herring (9) observed that temperatures below 16°C. (in air) were lethal for the cat, a finding confirmed by Britton in 1922. Hamilton (10) found that unanesthetized rats—which cool appreciably even in water at 30°C., according to Spealman—did not survive below an internal temperature of 12°C. (54°F.); life was 'precarious' up to 16°C., indeed, and for survival the use of artificial respiration often was required. In cats and kittens a colonic temperature of 75°F. (24°C) was reported as not critical but a reduction to 68°F. (18.5°C.) was definitely inimical. Ware, Hill and Schultz (11) very recently reported that in their cold-chamber experiments, newborn rats have recovered from colonic temperatures of 5°C.[5], 30-day old rats from 8°C., 60-day animals from 11°C. and 80-day old and up from 15°C. Two points of interest, especially, appear in their report: struggling only momentarily retarded rate of fall (room temperature 2° to 4°C.), and if respiration were impeded the fall was accelerated. Meader and Marshall (12) cooled mice in a dry-ice chamber at an ambient temperature of -10°C., with exposures of 25 and 65 minutes. Temperature decline was linear in character, and internal readings of 14.5°C., and as low as 8.5°C., were recorded. Some of the animals recovered spontaneously, others required artificial heat.

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[5] Lutz (Zeitschr. f. Kreislaufforsch. **36**: 625, 1944) reported recovery in the unanesthetized guinea pig from rectal temperatures of 3°C. by rapid warming in water at 50°C., with simultaneous artificial respiration and electrical stimulation of the heart.

Ariel, Bishop and Warren (13) reported that rabbits (anesthetized) could withstand reduction in iced bath to 20°C. with spontaneous recovery, provided the reduction was not too rapid, i.e. spanning not less than three to four hours. Reduction of the temperature below 20°C. rapidly diminished the capacity for recovery, although in some instances survivals occurred following depression to temperatures ranging from 17° to 10°C. Below 17°, however, additional heating usually was required; in the rewarming process, for example, of eight animals lowered to 10°C., four recovered with artificial heating, two recovered spontaneously, and two died despite the application of external heat.

The present report illustrates in a larger mammal the ability to withstand severe reductions in temperature, with recovery upon rapid rewarming and with no evident after-effects, and further illustrates the marked degree of bradycardia which can be tolerated. Moreover, loss of rigidity in the dog, under the conditions of the present experiments, is not the infallible index of irreversible cooling which it is reported to be in the human being (5), in which attempts at resuscitation, initiated during the flaccid state, were uniformly unsuccessful. In our experience, complete recovery was achieved in every instance from a stage of hypothermia considerably below the point at which muscle tone and reflex patterns were completely lost.

It seems probable that a severe hypoxia does not present a major problem to the hypothermic organism during the cooling process, in spite of an exaggerated A-V O_2 difference (5, 14) since under conditions of cooling one might reasonably anticipate a depression in the demand for oxygen. In favor of this is the observation that the survival time of the brain following interruption of its arterial supply is reported to be increased by cooling (W. Noell, cited by Alexander, 5). This circumstance, of course, may not hold true during the recovery period.

SUMMARY

The ability of normal dogs to withstand and to recover from marked cooling has been studied. Lightly anesthetized animals were submerged in iced water (2° to 9°C.) until a cardiac or respiratory crisis occurred. Resuscitation measures included rapid rewarming in water at 42° to 45°C., and artificial respiration as required.

Thirteen animals survived a reduction in deep rectal temperature to an average of 14.9°C. (range 11.7° to 16.8°C.). Recovery in each instance was complete. In two animals the rectal temperature was reduced to 15°C. or below on two occasions. Shivering did not appear to play an important rôle in the rewarming process; it set in at widely variant stages of recovery and varied in degree from mild to intense.

In cooling, the fall in body temperature was linear, a characteristic which in

its early phase probably was influenced by the pre-immersion anesthetic. The visceral-rectal temperature gradient became more pronounced as cooling proceeded. Comparison is shown between rate of cooling of the living and the dead body.

Decline in body temperature was accompanied by a progressive fall in arterial blood pressure and by a bradycardia which became intensified as temperatures fell below 20°C. The slow heart rate was uninfluenced by vagotomy or by atropinization.

Eight animals failed to respond favorably, or expired before rewarming was begun. Death was clearly a matter of respiratory failure in some; in others, it was apparently attributable to heart block. In one preparation, in which the rectal temperature fell to 11.5°C., 'rewarming death' occurred at a rectal reading of 18.2°C.

REFERENCES

1. Hemingway, A. Fed. Proceedings **6**: 128, 1947.
2. Spealman, C. R. This Journal **146**: 262, 1946.
3. Crismon, J. M. Mem. Rep. No. TSEAA-695-56A, Eng. Div. A.A.F. 21 Dec. 1945.
4. Burton, A. C. and H. C. Bazett. This Journal **117**: 36, 1936.
5. Alexander, L. Item No. 24, File No. XXVI-37, C.I.O.S., U. S. Army, July, 1945.
6. Gagge, A. P. and L. P. Herrington. Ann. Rev. Physiol. **9**: 409, 1947.
7. Grosse-Brockhoff, F. u. W. Schoedel. Arch. f. exper. Pathol. u. Pharmakol. **201**: 417, 1943.
8. Britton, S. W. Quart. J. Exper. Physiol. **13**: 55, 1922.
9. Simpson, S. and P. T. Herring. J. Physiol. **32**: 305, 1905.
10. Hamilton, J. B. Yale J. Biol. and Med. **9**: 327, 1937.
11. Ware, A. G., R. M. Hill and F. H. Schultz. This Journal **149**: 657, 1947.
12. Meader, R. G. and C. Marshall. Yale J. Biol. and Med. **10**: 365, 1938.
13. Ariel, I., F. W. Bishop and S. L. Warren. Cancer Res. **3**: 448, 1943.
14. Unpublished data.

OBSERVATIONS ON ENERGY METABOLISM AND WATER BALANCE OF MEN SUBJECTED TO WARM AND COLD ENVIRONMENTS[1]

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The opportunity to obtain data on energy metabolism and water balance occurred during experiments designed to determine the influence of environmental temperature on blood volume of men (1). It seems worthwhile to publish these results as a supplement to the growing and large, but probably still inadequate, volume of available information on these subjects. In the field of energy metabolism, discussions center about whether the resting, fasting metabolism of men is greatly increased (in the absence of shivering) in the cold and decreased in the heat, and whether the relative proportion of calorigenic substances (carbohydrate, fat and protein) metabolized is in any way influenced by ambient temperatures. Some recent reports (2, 3) suggest that a small increase in human metabolism may be present after several days of exposure to cold. Such results are in accord with the delayed response to cold of animals (rats and rabbits) in which a large metabolic increase can be induced (4, 5), but the degree of metabolic stimulation reported for the human subjects seems to be considerably less than has been stated in earlier literature (see 6). The metabolic mixture preferentially selected and burned is popularly believed to be influenced by environmental temperature. The results of at least one laboratory experiment (7) support the belief that diets of higher fat content are chosen by men exposed to cold. On the other hand, it has been demonstrated recently that a high fat diet (consisting solely of pemmican) is not immediately acceptable to soldiers during maneuvers under subarctic conditions (8).

Various conclusions have been reached with regard to environmental influence on water balance. Some investigators have been unable to demonstrate any consistent change in water reserves (7, 9, 10) as a result of altering environmental temperature, while in one study (11) exposure to heat seemed to lessen the water reserves. However, the belief persists (9) that the quantity of stored water is generally greater in warm than in cold environments.

EXPERIMENTAL METHODS

Resting, fasting metabolism was determined by collecting and analyzing expired air of subjects who breathed outside air (Tissot method). Caloric intake was estimated from weights of the various articles of food, using charts of food composition (12) and the usually employed caloric values of the food elements.

An approximate method for determining water balance, based upon the pro-

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[1] This investigation was supported by a Life Insurance Medical Research Fund Grant to Professor H. C. Bazett.

cedure described by Wiley and Newburgh (13), was employed. Total water intake was considered to be the sum of water drunk as such, water present in food and water arising from combustion. Water drunk as such was determined by weighing the water container of each individual at the beginning and end of the period. Water present in food was determined from weights of food using charts of food composition. Only an average estimate for metabolic water could be made. This was taken to be the quantity of water formed in the oxidation of the average daily diet. One gram of fat, carbohydrate and protein was assumed to produce respectively, 1.07, 0.60, and 0.41 grams of water.

Total water loss was considered to equal insensible water loss plus water of urine and feces. Water in urine was determined by subtracting the weight of solids from weight of urine. Weight of urinary solids was calculated from determinations of the specific gravity using Long's coefficient (14). Feces were assumed to contain 65 per cent water. Insensible water loss was obtained by subtracting from insensible weight loss the weight of carbon dioxide produced which is in excess of the weight of oxygen utilized. Differences in weights between carbon dioxide formed and oxygen utilized were calculated by multiplying average daily weight in grams of fat, carbohydrate and protein consumed by -0.06, $+0.4$, and $+0.08$, respectively.

Changes in amount of body tissue are neglected in these calculations. Since we were interested in demonstrating relatively large and sudden changes in water balance, this is presumably an unimportant consideration in our study in which caloric intake was fairly constant and approximately equal to caloric expenditure.[2]

OUTLINE OF EXPERIMENT

A detailed outline of the first two experiments has already been published (1). Briefly, two subjects, dressed in shorts, undershirts, and sandals, were studied in a controlled temperature room where they stayed continuously for periods of 9 to 14 days. In the first (summer) experiment, the room was maintained uncomfortably warm (33°C. D. B., 28°C. W. B.) for a period of four days. This was followed by a six-day period of cold (21°C. D. B., 16°C. W. B.) and a final four-day period in which the original warm conditions were reproduced. In the second (winter) experiment a cold period of two days was followed by seven days in the heat. The temperature of the cold and warm periods in the second experiment corresponded closely with those of the first. Wind velocity was approximately 50 cm. per second in all cases. Subjects were fed a mixed diet in unrestricted quantities. They were allowed complete freedom in requesting favorite articles of food, though a nutritionally adequate diet was presented each day. In actual practice, the cook exerted considerable influence on food selection since subjects often did not express preference. With the clothing worn (shorts and undershirts) subjects were near the shivering point during exposure to cold and uncomfortably warm during exposure to heat.

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[2] Wiley and Newburgh (13) overfed a normal human subject to the extent of 1600–1800 calories per day for a period of 15 days. Careful water balance studies showed a total gain of water during this period of only 897 grams though most of this was gained during the first few days. In comparison with this subject, over- or underfeeding of our subjects must have been of minor degree.

In a third experiment, four subjects were studied during several exposures of a single day to heat or cold. Each period began at 8 A.M. with subjects in the resting, fasting state and was terminated at the same time on the following morning. The subjects were then free for a day or two before another experimental period was begun.

RESULTS AND DISCUSSION

There is little evidence of any marked change in energy metabolism in the summer experiment (table 1). Elevated values for resting, fasting metabolism were recorded occasionally (in both cold and warm environments) following a few days of exposure to cold. (In *subject Y* there was only one value during this period which was above the range of values recorded up to that point.) The two-day period of cold in the winter experiment did not result in any very obvious trend in subsequent values for basal metabolism. Failure to stimulate metabolism in any great degree by the rather severe degree of exposure to cold supports the results of some of the more recent studies (2, 3) over those reported earlier (see 6). The discrepancy between our observations on men, and those made on animals (4, 5) in which a large (10 to 30 per cent) increase in basal metabolism is produced by a previous exposure to cold, may be due to the fact that the animals were subjected to a more severe degree of exposure. There is a suggestion in the present study and other recent studies (2, 3) that cold may cause a delayed stimulation of metabolism of man, as it does in animals.

Daily caloric intake was for the most part quite constant in each experiment, and was not obviously influenced by the temperature of the environment.[3] However, it does not necessarily follow that daily energy output remained unaffected by environment, for the subjects were not in exact metabolic balance. The composition of the diets during the warm periods of the summer experiment were: fat, 35–38 per cent; carbohydrate, 41–45 per cent; protein, 19–21 per cent. During the cold periods diets tended to be lower in fat (30–35 per cent) and higher in carbohydrate (46-50 per cent) with protein remaining at 19–21 per cent. Somewhat similar results were obtained in the winter experiment, the diets in the cold containing less fat and more carbohydrate and protein than the diets in the heat. The winter experiment is less satisfactory than the summer experiment for analysis, because of the scarcity of data during cold exposure. However, in each case the variation in proportion of dietary fat with exposure temperature seems to be correlated with the amount of milk consumed, i.e., milk was the article of diet which supplied the additional fat ingested in the heat. Milk consumed during periods of heat (both summer and winter) varied between 465 and 1106 grams per day; during cold exposure, milk consumption varied between 232 and 334 grams per day. This was in part due to the fact that subjects preferred a cool drink (milk) in the heat and a warm drink (hot tea) in the cold. The necessity for ingesting large quantities of fluid in the heat was probably also a

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[3] An abrupt change in caloric intake occurred in *subject R* toward the end of the summer experiment when his caloric intake fell by 15–20 per cent and remained at this lower level until the termination of the experiment. However, this change occurred during exposure to cold and continued throughout the ensuing period of heat; it was probably caused by an unhappy turn of events which occurred in the subject's personal affairs at about this time.

TABLE 1. DATA ON ENERGY METABOLISM OBTAINED DURING THE SUMMER EXPERIMENT OF 1946

Body weights in kgm. (wt.) were obtained at 10 P.M. (after urinating) using scales sensitive to a few grams. Basal metabolisms in Cal/m²/hr. (BMR) and respiratory quotients (R.Q.) were determined at 8 A.M. by collecting expired air (Tissot). Total daily caloric intake expressed in units of 1000 large calories (Tot. Cal.) and daily caloric intake of fat (Cal. fat), carbohydrate (Cal. Cho.) and protein (Cal. Prot.) were estimated from weights of food using standard charts of food composition. Room temperature during the warm periods was 33°C., D. B., 28°C., W. B.; during the cold periods, 21°C., D. B., 16°C., W. B. During the afternoon of the last day of each period, room temperature was changed; only observations completed before the temperature was changed are recorded.

DATE	SUBJ.													
	R							Y						
	Wt.	BMR	R.Q.	Cal. fat.	Cal. cho.	Cal. prot.	Tot. cal.	Wt.	BMR	R.Q.	Cal. fat	Cal. cho.	Cal. prot.	Tot. cal.
A. Warm														
15	61.2							52.8						
16	61.2			0.95	1.31	0.58	2.84	52.7	37.6	0.79	0.70	1.27	0.58	2.55
17	61.2	39.0	0.81	1.04	1.08	0.43	2.55	52.6			0.91	0.83	0.37	2.11
18	61.8			0.87	1.16	0.53	2.56	52.7	39.4	0.80	0.77	0.91	0.49	2.17
19		38.7	0.85											
Mean.....	61.4	38.9	0.83	0.95	1.18	0.51	2.65	52.7	38.5	0.80	0.79	1.00	0.48	2.28
B. Cold														
20	61.2			0.97	1.10	0.55	2.62	52.4	38.1	0.80	0.67	0.83	0.48	1.98
21	60.5	39.7	0.84	1.03	1.15	0.40	2.58	51.9			0.62	0.95	0.37	1.94
22	61.1			1.02	1.36	0.51	2.89	52.3	39.1	0.87	0.75	1.19	0.39	2.33
23	61.3	41.1	0.83	0.91	1.37	0.56	2.84	52.3			0.65	1.07	0.47	2.19
24	60.8			0.69	1.09	0.43	2.21	52.4	36.8	0.85	0.56	1.24	0.48	2.28
25		42.2	0.84											
Mean.....	61.0	41.0	0.84	0.92	1.21	0.49	2.63	52.2	38.0	0.84	0.65	1.06	0.44	2.14
C. Warm														
26	61.2			0.79	0.84	0.34	1.97	53.0	42.6	0.86	0.91	0.86	0.31	2.08
27	61.2	40.6	0.80	0.93	0.82	0.49	2.24	53.9			1.07	1.08	0.51	2.66
28	60.9			0.74	1.09	0.46	2.29	53.7	38.4	0.85	0.55	0.81	0.57	1.93
29		39.4	0.83											
Mean.....	61.1	40.0	0.82	0.82	0.92	0.43	2.17	53.8	40.5	0.86	0.84	0.92	0.46	2.22

factor in determining the quantity of milk consumed. It is not believed that heat caused any specific 'appetite' for fat, and this is supported by the fact that butter consumption was, if any significant changes occurred, decreased in the heat. These results do not disprove the general belief, based upon dietary habits of natives living in cold regions[4] and upon the results of other experiments (7), that diets high in fat are selected in cold environments. Since selection of diet at a given environmental temperature may be influenced by water contents of food available and by the temperature at which these foods are customarily served, it is not likely that human preferences for one or another of the calorigenic components of diet can be determined simply. A recent report on food selection of rats seems to demonstrate that carbohydrate is the variable element in dietary composition when caloric intake is altered by environmental temperature (15), but it cannot be assumed that these results on animals are applicable to man. Influence of environment on food selection is closely allied with the problem of altering the level of performance in environmental extremes by supplying diets of

TABLE 2. DATA ON ENERGY METABOLISM OBTAINED DURING THE WINTER EXPERIMENT OF 1946–47

This table is prepared in the same manner as table 1. Room temperature during the warm period was 33°C., D. B., 28°C., W. B.; and 21°C., D. B., 15°C., W. B. during the cold period.

DATE	SUBJ.													
	R							Y						
	Wt.	BMR	R.Q.	Cal. fat	Cal. cho.	Cal. prot.	Tot. cal.	Wt.	BMR	R.Q.	Cal. fat	Cal. cho.	Cal. prot.	Tot. cal.
							A. Cold							
26	59.5							51.3						
27	60.4			0.64	1.57	0.44	2.65	51.1	38.1	0.79	0.79	1.27	0.60	2.66
28		37.9	0.84											
Mean.....	60.0	37.9	0.84	0.64	1.57	0.44	2.65	51.2	38.1	0.79	0.79	1.27	0.60	2.66
							B. Warm							
29	61.9			0.72	1.35	0.30	2.37	52.8	39.2	0.81	1.17	1.34	0.54	3.05
30	61.8	36.4	0.77	1.03	1.37	0.43	2.83	52.5			0.81	1.11	0.28	2.20
31	62.5			0.81	1.15	0.39	2.35	52.8	40.8	0.84	0.81	1.32	0.39	2.52
1–1	62.4	37.1	0.86	0.72	1.73	0.34	2.79	52.9			0.54	1.29	0.28	2.11
1–2	63.0			1.11	1.46	0.55	3.12	52.5	37.5	0.80	0.82	1.10	0.49	2.41
1–3	62.6	39.2	0.88	1.16	1.27	0.39	2.82	52.9			1.18	1.12	0.36	2.66
1–4									37.7	0.79				
Mean	62.4	37.6	0.84	0.93	1.39	0.40	2.71	52.7	38.8	0.81	0.89	1.21	0.39	2.49

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[4] It may be noted that Greenland Eskimos are said to eat very little fat or blubber as such (18); however, certain parts of animals that are consumed, e.g., liver, are rich in fat.

different composition. Recent studies on human subjects indicate that performance in the cold is bettered by diets high in fat or carbohydrate over performance when diets high in protein are fed (16, 17). It may be noted that a smaller consumption of food energy (162 per cent of basal requirements) and a greater average weight loss (3.4 kgm.) occurred when diets high in protein were fed than occurred on feeding diets high in carbohydrate (191 per cent and 2.1 kgm. respectively). Relative undernourishment of subjects fed diets high in protein may have been partly responsible for differences in response to cold. A direct comparison between diets high in fat and diets high in protein was not made.

TABLE 3. DATA ON WATER BALANCE OBTAINED DURING THE WINTER EXPERIMENT OF 1946–47

Each period of water balance (B) covered the period from 10 P.M. one day to 10 P.M. the next. Water drunk (w) was determined by weighing water containers; water in food (f), from weights of food with the aid of charts of food composition; metabolic water (m), from daily food consumption (assuming metabolic balance). Water lost in urine (u) was determined from weights and specific gravities of urine, water in stools (s) was estimated from weights of stools; and insensible water losses (i) from insensible weight losses. All values are in grams. Room temperatures are given in table 2. On the days room temperature was changed, the first set of figures cover the period up to the time (5 P.M.) the temperature controls were adjusted, and the second set of figures cover the period from 5 P.M. to 10 P.M.

DATE	SUBJ.																	
	R									Y								
	Water intake				Water loss				B	Water intake				Water loss				B
	w	f	m	Tot.	u	s	i	Tot.		w	f	m	Tot.	u	s	i	Tot.	
A. Cold																		
12–27	0	2268	348	2616	888	0	870	1758	+858	128	1827	320	2275	1875	56	565	2496	−221
B. Transition from cold to warm																		
12–28	0	1245	275	1520	886	63	605	1554	−34	165	1115	253	1533	862	0	408	1270	+263
12–28	271	685	73	1029	115	50	295	460	+569	935	518	67	1520	151	211	268	630	+890
C. Warm																		
12–28	1719	1571	348	3638	665	0	1947	2612	+1026	1851	1954	320	4125	1155	96	2265	3516	+609
12–30	651	1863	348	2862	682	247	1948	2877	−15	1812	1374	320	3506	1637	98	2033	3768	−262
12–31	1537	1683	348	3568	753	0	2082	2835	+733	1619	1514	320	3453	715	96	2247	3058	+395
1–1	1505	1630	348	3483	1594	95	1888	3577	−94	1424	1364	320	3108	1066	84	1802	2952	+156
1–2	1655	1966	348	3969	903	366	2057	3326	+643	1536	1628	320	3484	1571	112	2129	3812	−328
1–3	1011	1892	348	3258	1193	185	2111	3489	−231	1433	1650	320	3403	721	137	2149	3007	+396
D. Transition from warm to cold																		
1–4	700	1084	275	2059	874	68	1759	2701	−642	736	921	253	1910	696	0	1817	2513	−603
1–4	184	697	73	954	—	325	202	—	—	70	686	67	823	295	129	149	573	+278

Both subjects gained weight in the winter experiment (table 2) and this was associated with approximately an equal gain in water as judged by water balance studies (table 3). The close agreement may be fortuitous for the daily water balance is inaccurate because of error introduced by the assumption of metabolic balance. Summation of these errors over a period of many days may indicate a change in a certain direction in water balance whereas in reality the opposite may have occurred. The daily water balances indicate that water was retained in large quantities during the first 29 hours of exposure to heat (from 5 P.M. on 12-28 to 10 P.M. on 12-29). Since the period from 5 to 10 P.M. on 12-28 is a period in which water normally seems to be stored (probably because of the evening meal), the significance of water retention during this time in our experiments may be doubtful. However there remains the fact that water retention of large magnitude occurred during the subsequent day. The quantities involved are too great to result from formation of new tissue; and presumably the water was stored in extracellular spaces. A causal relationship between warming the environment and increasing body water content is, of course, not established by this single study. Furthermore, the experiment was not suitably designed to demonstrate water loss on exposure to cold. In this particular experiment, the initial exposure to cold was complicated by a change in the general living condition of the subjects, i.e., subjects were not only exposed to a temperature change during the single day of cold for which data for water balance are available, but they also began living in the controlled temperature room. In the final cold period, data on water balance for only a fraction of a day were obtained.

TABLE 4. DATA ON WATER BALANCE OBTAINED IN SEVERAL EXPERIMENTS OF ONE-DAY DURATION (FROM 8 A.M. TO 8 A.M.)

Water balances were obtained in the manner illustrated in table 3. Room temperature was 33°C., D. B., 29°C., W. B. in the warm periods and 21°C., D. B., 18°C., W. B. in the cold.

DATE	SUBJ.							
	A		B		D		K	
	Warm	Cold	Warm	Cold	Warm	Cold	Warm	Cold
7/21	+674		−403					
7/24		−173		−207				
7/26	+261		−50		+1830			
7/31	+174		+408		+772		+224	
8/2		−292		−431		+362		−429
8/5	+513		−279		+549		−1	
Means.....	+406	−233	−162	−319	+788	+362	+112	−429

In the summer experiment body weights of both subjects decreased on exposure to cold and increased on exposure to heat. In one subject (*R*) transfer from cold to heat took place very soon after caloric intake of this subject dropped sharply, but even in this case average body weight during exposure to cold was less than during either period of heat exposure. These changes in weight, for the most part, are presumably too large and abrupt to be caused by caloric imbalance, i.e., formation or destruction of body tissue. The data on water balance in this experiment are not presented since records were not kept with sufficient accuracy to be reliable.

The water balance studies made during exposure for only one day to heat or cold (table 4) are also complicated by the change from home life to living in the controlled temperature room. On the average, however, the influence of environmental temperature was apparent in each subject. Two subjects (*A* and *K*) exhibited a positive water balance during exposure to heat and a negative balance in the cold. Subject *D* gained water under both conditions but gained more in the heat and the remaining subject (*B*) lost water both in the heat and cold but on the average lost more in the cold.

All considered, our results are fairly consistent in showing an effect of environmental temperature on water storage. Burton *et al.* (7) obtained equivocal evidence of similar changes in their experiments which were thought to be complicated by changes in amount of body tissue. Conley and Nickerson (10) found in two of four experiments a decrease in thiocyanate space on transferring subjects from heat to cold; in the other two experiments, and in two additional experiments in which subjects were transferred from cold to heat, a significant change in this fluid compartment was not found. Their error of measurement was estimated at three per cent, which would amount to about one-half liter in an individual of average size. Forbes *et al.* (11) found an average decrease in interstitial fluid of 11 per cent in a group of ten individuals transferred from Massachusetts to Mississippi. However, the variation among individuals was from −34 to +26 per cent. Apparently extraneous factors must have been present to account for such large fluctuations in water reserves.

SUMMARY

1. Studies of energy metabolism and water balance were made on two subjects living in a controlled temperature room. In one experiment, a warm period (33°C., D. B., 28°C., W. B.) of four days was followed by six days of cold (21°C., D. B., 16°C., W. B.) and a terminal period of four days of heat. A second experiment consisted of two days of cold followed by seven days of heat and a final brief period of cold. Four other subjects were studied in a series of experiments consisting of exposure for a single day to either heat or cold.

2. Energy metabolism was not affected greatly by these environmental extremes as judged by records of caloric intake, dietary composition, basal metabolism and resting, fasting respiratory quotients. Values for basal metabolism in one experiment were on the average slightly higher following exposure to cold for a few days than they were prior to exposure, but the differences are small. Diets consumed in the heat contained somewhat more fat and less carbohydrate or protein than diets consumed in the cold. The increased milk consumption, which was responsible for the additional dietary fat in the heat, did not seem to be the result of any specific 'appetite' or 'desire' for fat.

3. Evidence for storage of water on changing from a cold to a warm environment and loss of water under opposite circumstances was obtained from water balance studies and observations of body weight. The quantities of water involved ranged from a few hundred cubic centimeters to more than a liter.

REFERENCES

1. SPEALMAN, C. R., MICHAEL NEWTON AND R. L. POST. This Journal **150:** 628, 1947.
2. HORVATH, STEVEN M., A. FREEDMAN AND H. GOLDEN. This Journal **150:** 99, 1947.
3. NEWBURGH, L. H. AND C. R. SPEALMAN. C. A. M. Report No. 241, 15 December 1943.
4. SCHWABE, E. L., F. E. EMORY AND F. R. GRIFFITH. J. Nutrition **15:** 199, 1938.
5. LEE, R. C. J. Nutrition **23:** 83, 1942.
6. SWIFT, RAYMOND W. J. Nutrition **5:** 227, 1932.
7. BURTON, A. C. *et al.* This Journal **129:** 84, 1940.
8. KARK, R. M., R. E. JOHNSON AND J. S. LEWIS. War Med. **7:** 345, 1945.
9. BAZETT, H. C. *et al.* This Journal **129:** 69, 1940.
10. CONLEY, C. LOCKHARD AND JOHN L. NICKERSON. This Journal **143:** 373, 1945.
11. FORBES, W. F., D. B. DILL AND F. G. HALL. This Journal **130:** 739, 1940.
12. CHATFIELD, CHARLOTTE AND GEORGIAN ADAMS. U. S. Dep. Agriculture Circular No. 549, June 1940.
13. WILEY, F. H. AND L. H. NEWBURGH. J. Clin. Inv. **10:** 723, 1931.
14. BODANSKY, MEYER. Introduction to Physiological Chemistry. John Wiley and Sons, Inc., New York, Fourth Edition, 1938. P. 449.
15. DONHOFFER, SZ. AND J. VONTOZKY. This Journal **150:** 329, 1947.
16. KEETON, ROBERT W. *et al.* This Journal **146:** 66, 1946.
17. MITCHELL, H. H. *et al.* This Journal **146:** 86, 1946.
18. THOMAS, WILLIAM A. J. Am. Med. Assoc. **88:** 1559, 1927.

REACTIONS OF MEN EXPOSED TO COLD AND WIND

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The contribution made by outer garments of low air permeability to the overall insulative value of cold-weather garments and to the thermal balance and comfort of the wearer is uncertain, although no one doubts either the value or the necessity of an outer windproof layer. Since the increased heat losses which occur with wind arise primarily from air exchange within the clothing, the thicker and the more permeable the under garments, the greater is the necessity for a good windproof. It has been suggested that the degree of windproofing required depends upon the relative permeability of the underlying layers. If the layer most immediate to the windproof outer garment offers some resistance to air movement, the increased heat loss by convection and air exchange with wind is consequently diminished. This can be illustrated by comparing thermal experiences under the two conditions of use of the alpaca-mohair pile garment of the Arctic issue. If the pile is placed outside, next to the windproof garment, greater heat losses occur with wind than when the backing of the pile is worn adjacent to the outer garment.

Troops, especially mechanized units, operating in regions of extreme cold are faced with the problem of both cold and wind. Siple's (1) data on wind chill and the reports of Horvath *et al.* (2) on men exposed to cold without wind could not be extended to these conditions. Since the data available were inadequate to determine either the degree of stress or the type of response that would occur in men exposed to cold and air movement, studies were conducted on soldiers seated quietly in environments where both temperature and wind velocity were controlled.

METHODS

These experiments were conducted in the laboratory cold room for the most part at an environmental temperature of −23°C. Other temperatures employed were −17° and −36°C. Previous experience with the Arctic issue had demonstrated that at −17°C. without air movement men could sit quietly in relative comfort for periods of four to five hours. However, exposure at an ambient temperature of −17°C. would necessitate rather long experimental periods and other factors such as hunger, boredom, etc. might become deciding elements. It was also known that the Arctic issue offered adequate protection for two to three hours at −29°C. Therefore, it was felt that an intermediate temperature of −23°C. would enable differences to be detected without any of the disadvantages of the lower or higher temperatures.

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[1] This data was obtained while the author was at the Armored Medical Research Laboratory, Fort Knox, Ky.

A tunnel 18 feet long, 7 feet high, and 5 feet wide was constructed in the cold room. Six fans were so placed that when air was drawn through the tunnel turbulence was minimal. The tests were conducted at three wind velocities: 0, 5, and 9.6 mph. By placing two subjects on tables, four men could be exposed simultaneously to an uninterrupted air stream. In all of the tests a copper cylinder, wrapped in the four layers of the complete Arctic issue, was suspended from the roof of the tunnel in the midst of the subjects, and the rate of cooling of the contained water was measured.[2] This physical method, employed as a measure of environmental stress, indicated that constant conditions were maintained during the experiments.

Ten representative subjects were selected from a group of 50 men whose subjective responses to cold had been previously determined. Originally, of the five subjects selected for detailed study, three were classified as resistant and two as susceptible to cold. Unfortunately, considerable difficulty was experienced in outfitting the men, so that four men who had been tentatively classified susceptible and only one resistant were finally employed. During the progress of the test, however, it became evident that this classification of relative resistance was uncertain and was subject in the majority of cases to considerable daily variation, so that the failure to have equality at the outset in number of 'resistants' and susceptibles' was not very important.

A standardized procedure was followed prior to each day's test. The men arrived at 0645 hours, ate a standard breakfast, and then rested until they dressed for the test which began at 0830 hours. Tests were conducted both morning and afternoon but only those data obtained during the morning experiments are presented here, since the subjects' reactions differed somewhat from morning to afternoon.

Skin temperatures were obtained on chest, upper arm, thigh, calf, and dorsal surface of the foot of each of the four subjects in the tunnel. In some cases ten copper constantan thermocouples were worn. Continuous measurements of oxygen consumption were made on two of the subjects, one with an open-circuit and the other with a closed-circuit apparatus. Rectal temperatures were obtained by clinical thermometers. The clothing worn by the subjects is listed below.[3] A complete set of tests on all subjects in each of the windbreaks was impossible. Nevertheless, a sufficient number of tests was made on all three of these clothing items, with at least two subjects wearing each of the items, so that general conclusions may be drawn.

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[2] This device was suggested by Dr. Theodore Hatch.

[3] Standard Arctic clothing worn by subjects consisted of the following: drawers, wool, 50/50; undershirts, wool, 50/50; shirt, flannel, O.D.; trousers, field, wool, O.D.; trousers, field, pile; trousers, field, cotton, O.D. (sateen, 9 oz.) windproof; parka, field, cotton, O.D. (sateen, 9 oz.) windproof; parka, pile; shoe, Arctic, felt; socks, wool, ski (2 pairs); socks, wool, cushion sole (1 pair); mittens, insert, trigger finger M-1943; mittens, shell, trigger finger M-1943; mufflers, wool; and wristlets, knit.

Three types of windbreak materials for the outer garments were used: *a*) nylon-air permeability of 0 cu. ft/sq. ft/min.; *b*) sateen, 9 oz.—air permeability of 5 cu. ft/sq. ft/min. (this is identical to the material used in the control parka and trousers); and *c*) herringbone twill-air permeability of 20 cu. ft/sq. ft/min.

RESULTS AND DISCUSSION

Effect of wind upon insulation. Data on the insulative value of the standard Arctic clothing, the sateen outer garments having an air permeability of 5 cu. ft/sq. ft/min., are presented in table 1. Hourly Clo values, determined by the standard procedure (3, 4) are presented in the table together with the average Clo values for the second and third hours. The Clo values as determined from the third-hour metabolic rate and the predicted equilibrium temperature, θ_e, are also given for the five subjects (5). All values are corrected for the insulation of the surrounding air. During the three-hour exposure, the apparent insulation of the Arctic issue was changing; it appeared to increase with time under still-air conditions. This was true to a lesser extent at 5 mph. wind. But with an air movement of 9.6 mph., there was, after the first hour, a fairly consistent decrease in insulative value with increasing wind velocity.

TABLE 1. INSULATIVE VALUE OF THE STANDARD ARCTIC CLOTHING IN RELATION TO WIND VELOCITY

SUBJECT	WIND VELOCITY	INSULATION				
		Clo Units, by standard method				Clo Units by θ_e
		First hour	Second hour	Third hour	Average of 2nd and 3rd hours	
	mph.					
GO	0	3.1	3.4	—	3.4	5.2
	5	4.3	3.9	5.5	4.7	5.0
	9.6	2.7	3.1	4.1	3.6	4.0
HE	0	3.5	5.7	3.8	4.7	5.3
	5	2.9	4.3	3.2	3.8	4.5
	9.6	2.7	3.8	2.8	3.3	3.7
GR	0	4.1	4.3	5.1	4.7	4.9
	5	3.6	4.2	4.4	4.3	4.2
	9.6	3.3	3.5	2.8	3.2	3.7
VA	0	3.8	5.0	4.8	4.9	5.0
	5	3.2	5.0	3.8	4.4	5.2
	9.6	3.5	4.5	4.3	4.4	5.5
BA	0	2.5	4.3	3.0	3.7	3.9
	5	2.3	4.8	4.2	4.5	4.1
	9.6	5.2	2.9	3.7	3.3	4.1
Average for all subjects........	0	3.5	4.5	4.2	4.4	4.9
	5	3.4	4.4	4.2	4.3	4.6
	9.6	3.5	3.5	3.5	3.5	4.2

No material differences in the insulation afforded by the Arctic clothing modified by substitution of outer garments having different air permeabilities were noted at zero wind velocities (table 2). When the wind velocity was increased to

5.0 and 9.6 mph., the Clo values decreased in all cases except when the nylon outer garment was worn. The high average insulation at 9.6 mph. in this case, however, resulted from an extreme value, 7.0, on one of the two subjects, while in the other the insulation value was lower than at either 5.0 or 0 miles per hour. The greatest loss of insulation with wind occurred with the herringbone twill wind-proof, the average Clo value dropping from 5.3 to 3.6.

TABLE 2. INSULATIVE VALUE OF STANDARD ARCTIC CLOTHING IN RELATION TO THE AIR PERMEABILITY OF THE OUTER GARMENT LAYER

(Average values from two to six subjects in Clo units, calculated from θ_e)

ARCTIC CLOTHING WITH OUTER GARMENTS OF THE FOLLOWING WINDPROOF MATERIALS	WIND VELOCITY, MILES PER HOUR		
	0	5.0	9.6
Nylon[1]	5.3	4.3	5.8
Sateen—9 oz	4.9	4.6	4.2
Herringbone twill	5.4	4.3	3.6

[1] Two subjects.

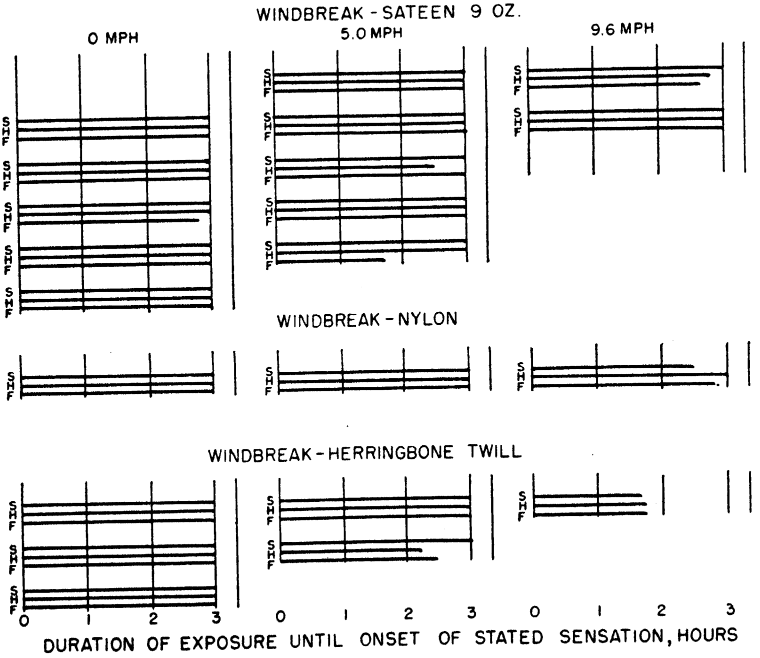

FIG. 1. SUBJECTIVE REACTIONS of *Subject HE* exposed at −23°C. to varying wind velocities while dressed in Arctic issue and different windbreaks. F = onset of pain in feet; H = onset of pain in hands; S = onset of shivering. Extension of line to 3 hrs. indicates no stated sensation during exposure.

The decrease in the apparent insulative value of the clothing assembly with increasing air movement no doubt resulted from interference with the stability of the bound air layer. Insulation of clothing is essentially determined by the thickness and stability of the air immobilized between fabric layers and within the fabrics themselves. There is a possible three-fold variation in the insulation from a completely static air layer to one within which there is free convection. At high-wind velocities two factors may be operating to alter the insulation: circulation within the clothing and internal circulation plus air exchange with the outside. Both may result in real increases in heat transfer.

Effect of wind on body-cooling rate. The advantage of windproof garments is demonstrated by the mean skin temperature curves in figure 4. This subject VA, was exposed to −17°C. for a period of five hours with and without air movement. The slightly greater decrease in the mean skin temperature at the 5 mph. wind was evident from the very beginning. This initial rapid fall was stabilized quickly during the remainder of the experiment. In one of his repeat exposures to wind and cold, VA inadvertently made an 18-inch linear tear in the front of his parka. Since such events commonly occur, it was decided to continue the experiment. The mean skin temperature fell rapidly and continued to levels more commonly observed at much colder ambient temperatures. The subject complained very early about cold, discomfort and shivering. He was unable to continue for more than four hours.

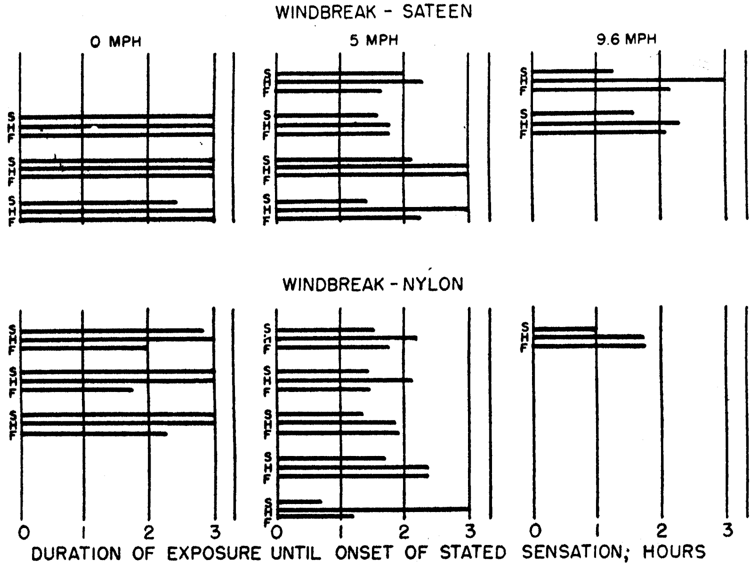

FIG. 2. SUBJECTIVE REACTIONS of *Subject GR* exposed at −23°C. to varying wind velocities while dressed in Arctic issue clothing modified by two different types of windproof garments. For explanation see fig. 1.

The data on mean skin and rectal temperatures are summarized in table 4. Neither the values of the cooling constant, K, the mean skin temperature at the end of the exposure period nor the predicted equilibrium temperature, θ_e, exhibited great change with the type of outer garment being worn by these men. However, slight trends in the average data relative to wind velocity were observed. The median values for the last mean skin temperature recorded were 26.0°, 25.2° and 24.8°C. at wind velocities of 0.0, 5.0 and 9.6 mph., respectively. The predicted equilibrium mean skin temperatures followed this same pattern but were approximately 1.2°C. lower. The median values for *K*, the cooling constant, were 0.720, 0.755 and 0.835 for the three wind velocities. These differences are not significant.

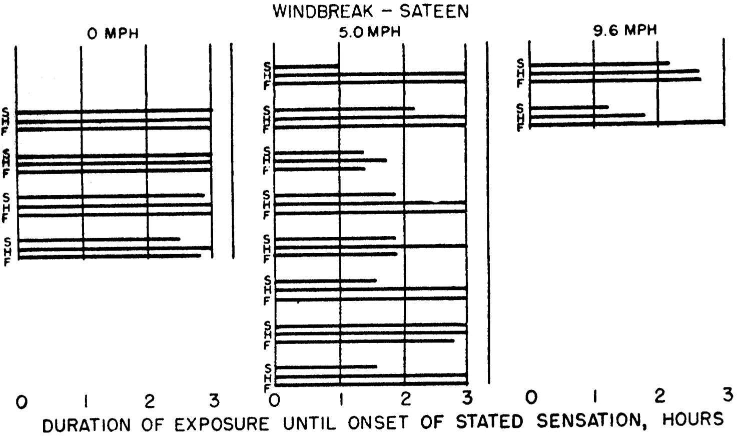

FIG. 3. SUBJECTIVE REACTIONS of *Subject VA* exposed at −23°C. to varying wind velocities. For explanation see fig. 1.

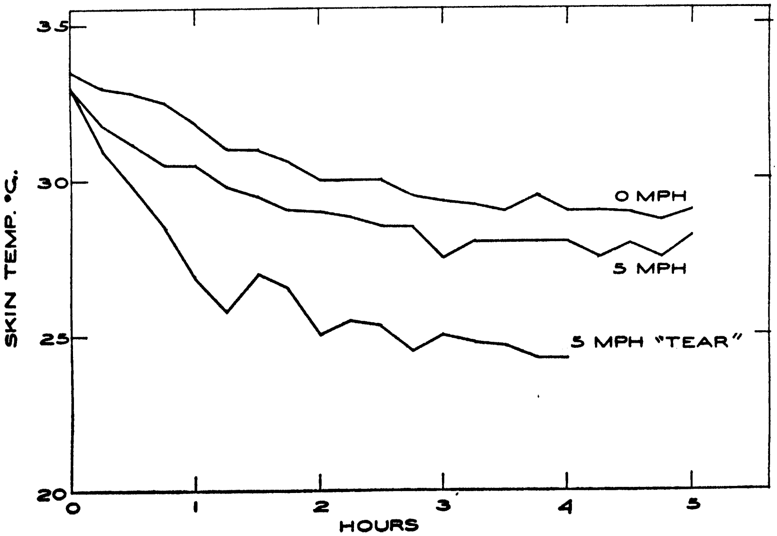

FIG. 4. MEAN SKIN TEMPERATURES of *Subject VA* exposed to −17°C., illustrating the influence of wind and loss of protection as a consequence of an 18-inch tear in the windproof parka.

The fall in rectal temperature was not appreciably affected by either the ambient temperature or the wind velocity. The principal effect of these environmental conditions was reflected in the changes in mean skin temperatures. Although it appeared possible to generalize from these values and obtain information regarding equivalent environments such as have been developed for hot climates by Robinson *et al.* (6), it was felt best not to do so in view of the present state of our knowledge regarding exposure of men to cold.

The alterations in metabolic rate with low ambient temperature, air movement and the relative permeability of the outer garments were significant. Table 3 presents such data for the standard Arctic assembly with the total caloric output corrected for heat lost both in warming the inspired air and by evaporation of water from the lungs. The metabolic response of the subject under these experimental conditions was approximately the same for the first hour at all wind velocities. During the second and third hours, however, a marked stimulus for extra heat production was evident. The higher the wind velocity, the sooner was the stimulus to extra heat production developed and the greater the response. In this connection, it is noteworthy that, as a rule, the greatest changes in stored heat occurred during the first hour. Variable but generally much smaller demands on this heat supply were made during the next two hours. Neither the absolute amount nor the rate of the heat loss from storage appear to correlate with the stimulus to extra heat production.

TABLE 3. HOURLY METABOLIC RATES[1] IN RELATION TO WIND VELOCITY STANDARD ARCTIC CLOTHING

(Averages for five subjects)

	0 MPH		5.0 MPH		9.6 MPH	
	Cals/M²/hr.	Per cent increase	Cals/M²/hr.	Per cent increase	Cals/M²/hr.	Per cent increase
First hour	38.3		39.0		39.4	
Second hour	42.7	11	45.2	16	63.6	61
Third hour	51.3	34	60.1	54	67.8	73

[1] Corrected for heat lost in warming inspired air and water evaporation from lungs.

TABLE 4. MEAN SKIN AND RECTAL TEMPERATURES OBTAINED DURING THE SITTING EXPOSURES TO VARIOUS ENVIRONMENTS AND WIND VELOCITIES

ENVIR. TEMP.	WIND VELOCITY	DURATION OF EXPOSURE	INITIAL SKIN TEMP.	FINAL SKIN TEMP.	Δ SKIN TEMP.	INITIAL RECTAL TEMP.	FINAL RECTAL TEMP.	Δ RECTAL TEMP.
°C.	*mph.*	*hours*	°C.	°C.	°C.	°C.	°C.	°C.
−23	0.0	3	33.0	25.0	−7.0	37.3	36.8	−0.5
−23	5.0	3	33.1	25.2	−7.9	37.4	36.8	−0.6
−23	9.6	3	32.4	24.8	−7.6	37.3	36.7	−0.6
−17	0.0	5	33.1	27.3	−5.8	37.3	36.7	−0.6
−17	5.0	5	32.6	25.0	−7.6	37.3	36.6	−0.6
−36	0.0	3	33.3	24.7	−8.6	37.4	36.9	−0.5

Men dressed in the nylon or sateen windproof garments had similar metabolic responses to increased air movement and duration of exposure. The herringbone twill garments offered less protection against wind, however, and the extra heat production of subjects dressed in these was greater than with the others, the hourly metabolic rates being 42, 93 and 85 Cal/M^2/hr.

Subjective responses. The subjective responses of the men were significantly altered when they were exposed to cold and wind (fig. 1, 2, and 3). *Subject HE*, figure 1, was the most resistant subject in this study. When he wore adequate outer windproof garments (sateen or nylon), *HE* did not become uncomfortable even at the highest wind velocity. In herringbone twill outers, however, he failed to maintain comfort at 5.0 mph., and at a wind velocity of 9.6 mph., he was definitely uncomfortable. For the less resistant subjects, the herringbone twill was equally inadequate at 5.0 mph. wind. In figure 2 are recorded the subjective sensations for *Subject GR* in the two best windproof outer garments, sateen and nylon. He remained relatively comfortable at zero wind velocity, but with air movement his subjective sense of discomfort increased. *GR* appeared to be less comfortable when wearing the nylon than with the sateen outer garment. This is in striking contrast to the subjective experiences of *Subject HE* as shown in figure 1.

Exposure of men to rapid air movements diminishes the time from initial exposure to the appearance of sensations of discomfort. Although general body comfort, as evidenced by onset of shivering, appears to be most readily influenced by wind, the onset of discomfort in the extremities does not lag very far behind. Shivering, continued for any length of time, is a distressing experience, fatiguing and distracting. Fine muscular control is almost impossible and concentration is difficult, one thought dominating—the desire for termination of shivering. Under such conditions, the general overall efficiency is impaired and the necessity for adequate protection is quite clear.

The author wishes to express his appreciation to the men who so willingly subjected themselves to the tests and to Mr. James Gregg, Sgt. H. Golden, my wife, Elizabeth, and Mrs. James M. Nelson for their technical assistance.

REFERENCES

1. Siple, P. General Principles Governing Selection of Clothing for Cold Climates. Report to the Office of the Quartermaster General, June 1942.
2. Horvath, S. M., H. Golden and J. Wagar. Journal Clin. Invest. **25**: 709, 1946.
3. Gagge, P. A., A. C. Burton and H. C. Bazett. Science **94**: 428, 1941.
4. Belding, H. S., R. C. Darling, D. R. Griffin, S. Robinson and E. S. Turrell. Clothing Test Methods, pp. 9–20. National Research Council, 1945.
5. Hatch, T. Communication to the Armored Medical Research Laboratory, 1944 (available upon request).
6. Robinson, S., E. S. Turrell and S. D. Gerking. This Journal **143**: 21, 1945.

EFFECTS OF ACUTE EXPOSURE TO SIMULATED ALTITUDES ON DEXTROSE TOLERANCE AND INSULIN TOLERANCE

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Although there have been numerous investigations of the effects of anoxia on fasting blood glucose levels, there is little data in the literature with regard to the effects of altitude on dextrose tolerance and insulin tolerance. Most investigators have reported that anoxia causes hyperglycemia (1, 5, 6, 7, 11, 13, 14, 15, 17, 18, 19) although some have reported that anoxia has no effect on fasting blood sugar (8, 10), and some have reported that even hypoglycemia may result under certain conditions from anoxia (16, 21). Middlesworth, Kline and Britton (20) have recently demonstrated that blood sugar levels may either rise, fall or remain unchanged under anoxia, depending upon the absorptive condition of the animal, length and degree of anoxic exposure and degree of acclimatization.

There are also contradictory reports in the literature regarding the effects of altitude on dextrose tolerance, which was found to be decreased (2), increased (8), and unchanged (3, 4, 16). . D'Angelo (4) found a difference in the hypoglycemic component of the glucose tolerance curves between altitude and ground level, with the depression of blood sugar values below the pre-ingestion value being of greater magnitude and duration at ground level. This difference, however, could not be correlated with any difference in severity or duration of hypoglycemic attack. Reports on the effect of insulin on blood sugar levels under conditions of anoxia (10, 18, 19) are quite divergent as regards the incidence, type and severity of insulin convulsions observed, but they are in agreement on the fact that blood sugar levels obtained are no lower than those following similar doses of insulin at atmospheric pressure. We have found no reports of dextrose tolerance tests performed on unacclimatized animals immediately upon acute exposures to altitude anoxia, nor of standard insulin tolerance tests made under similar conditions. In the present study we have performed these two types of experiments.

METHODS

The animals used in these experiments were three well-trained male Dalmatian coach hounds, which were litter mates of a carefully inbred, pedigreed strain.

The dogs were loosely restrained in a supine position on animal boards. No anesthesia was used and all blood samples were obtained by external jugular puncture. The dogs were fasted for twelve hours prior to the tests.

In all experiments conducted under reduced atmospheric pressure, a standard procedure was adhered to under which the dog was restrained on the animal board in the altitude chamber and ascent to the desired altitude was accomplished at the rate of 2000 feet per minute. Within five minutes after arrival at the desired altitude the fasting blood sample was drawn. In order to minimize the acclimatization factor, an interval of at least one week was maintained between successive exposures of any dog to altitude.

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For the intravenous dextrose tolerance tests, the test dose of dextrose used was 0.5 gram per kilogram of body weight. Blood samples were drawn immediately before and 30 minutes, one hour, 2 hours and 3 hours after the injection of the test dose.

The insulin tolerance test was done as described by Fraser, Albright and Smith (9) with the exception that .2 unit of insulin and .02 cc. of 1:1000 adrenalin per kilogram of body weight were used. Blood samples were drawn immediately before and 20 minutes, 30 minutes, 45 minutes, one hour, one hour and 30 minutes, and 2 hours after the intravenous injection of insulin. Immediately after the two-hour sample was drawn, the calculated amount of adrenalin was injected intramuscularly. Two more blood samples were then drawn at 45 and 60 minutes after this injection.

All blood sugar determinations were done by the Horvath and Knehr (12) method.

RESULTS

The results obtained with the dextrose tolerance tests at ground level[2], 18,000 feet and 24,000 feet are shown in table 1 and figure 1. Table 1 gives both the absolute blood sugar levels and the absolute changes from the initial (fasting) levels. The curves shown in figure 1 are plotted on the basis of the absolute changes in blood glucose level as compared to the initial (fasting) level, taken as zero.

It will be noted from these data that there are definite alterations in form of the dextrose tolerance curves at altitudes of 18,000 and 24,000 feet, respectively, as compared to the baseline values done at ground level[2]. Although these alterations are not strictly parallel quantitatively from dog to dog, they are quite similar qualitatively as regards the general trends of the curves. For example, it is obvious from observation of these curves that all of the curves of the individual animals as well as the mean curve for the group show: 1) at 18,000 feet a 30-minute value approximately one half as high as the corresponding value at ground level, but at 24,000 feet a 30-minute value approximately of the same magnitude as the corresponding value at ground level; 2) at 18,000 feet a slower rate of fall of blood glucose level, so that in spite of the low 30-minute values, the one-hour levels are in every case higher than the one-hour values at ground level and at 24,000 feet an even slower rate of fall during the corresponding period; (in one case a marked rise in the blood sugar level appeared at one hour as compared to the 30-minute value) and 3) a failure of the glucose level to return to initial levels, either at 18,000 feet or at 24,000 feet in the three-hour period.

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[2] Ground level as used here means 'ground level at Randolph Field, Texas, (approximately 750 feet)'.

Changes in insulin tolerance were not as marked as those in dextrose tolerance. Table 2 and figure 2 show the results of the insulin tolerance tests. It will be noted that the minimum levels of blood glucose obtained at altitudes of 18,000 feet and 24,000 feet were approximately the same and were not as low as those obtained at ground level. Furthermore, the response to adrenalin in *dogs no. 1* and *no. 2* was decidedly less marked at altitudes of 18,000 feet and 24,000 feet than at ground level.

It is interesting to note that the fasting blood sugar values for blood taken approximately five minutes after arrival at altitudes of 18,000 feet and 24,000 feet, respectively, are altogether comparable with the fasting values obtained at ground level.

TABLE 1. DEXTROSE TOLERANCE TESTS

ALTITUDE	TIME IN HRS.	BLOOD GLUCOSE LEVEL (MGS. PER 100 CC.)			BLOOD GLUCOSE LEVEL (EXPRESSED AS ABSOLUTE CHANGE FROM INITIAL FASTING LEVEL)			
		Dog 1	Dog 2	Dog 3	Dog 1	Dog 2	Dog 3	Average
Ground level	0	106	120	132	0	0	0	0
	½	190	212	199	84	92	67	81
	1	130	135	111	24	15	−21	6
	2	112	123	128	6	3	−4	2
	3	114	128	142	8	8	10	9
18,000 ft.	0	125	125	122	0	0	0	0
	½	171	168	154	46	43	32	40
	1	168	143	125	43	18	3	21
	2	131	145	143	6	20	21	16
	3	137	144	134	12	19	12	14
24,000 ft.	0	139	125	122	0	0	0	0
	½	227	199	177	88	74	55	72
	1	240	160	176	101	35	54	63
	2	282	155	171	143	30	51	75
	3	201	149	192	62	24	70	52

DISCUSSION

Although the data presented demonstrate considerable variation from animal to animal in response to altitude anoxia, certain trends are quite striking. These are 1) a decreased hyperglycemia responsiveness, more marked at 24,000 feet than at 18,000 feet, 2) an increased insulin resistance, 3) a decreased response to adrenalin and 4) a uniformly low 30-minute blood sugar level at 18,000 feet. This latter finding is difficult to explain since it would indicate an increased hyperglycemia responsiveness during the first 30 minutes following injection of dextrose in spite of a decreased hyperglycemia responsiveness in the remainder of the period studied.

It is interesting to note that the fasting blood sugar values obtained approximately five minutes after arrival at simulated altitudes of 18,000 feet and 24,000 feet, respectively, are altogether comparable with the fasting levels obtained on these same animals at ground level. This strongly suggests that at this point in exposure to reduced oxygen tension the sympathico-adrenal activity is not demonstrably increased, and one must therefore be cautious in invoking this mechanism to account for elevated blood sugar frequently noted under anoxic conditions.

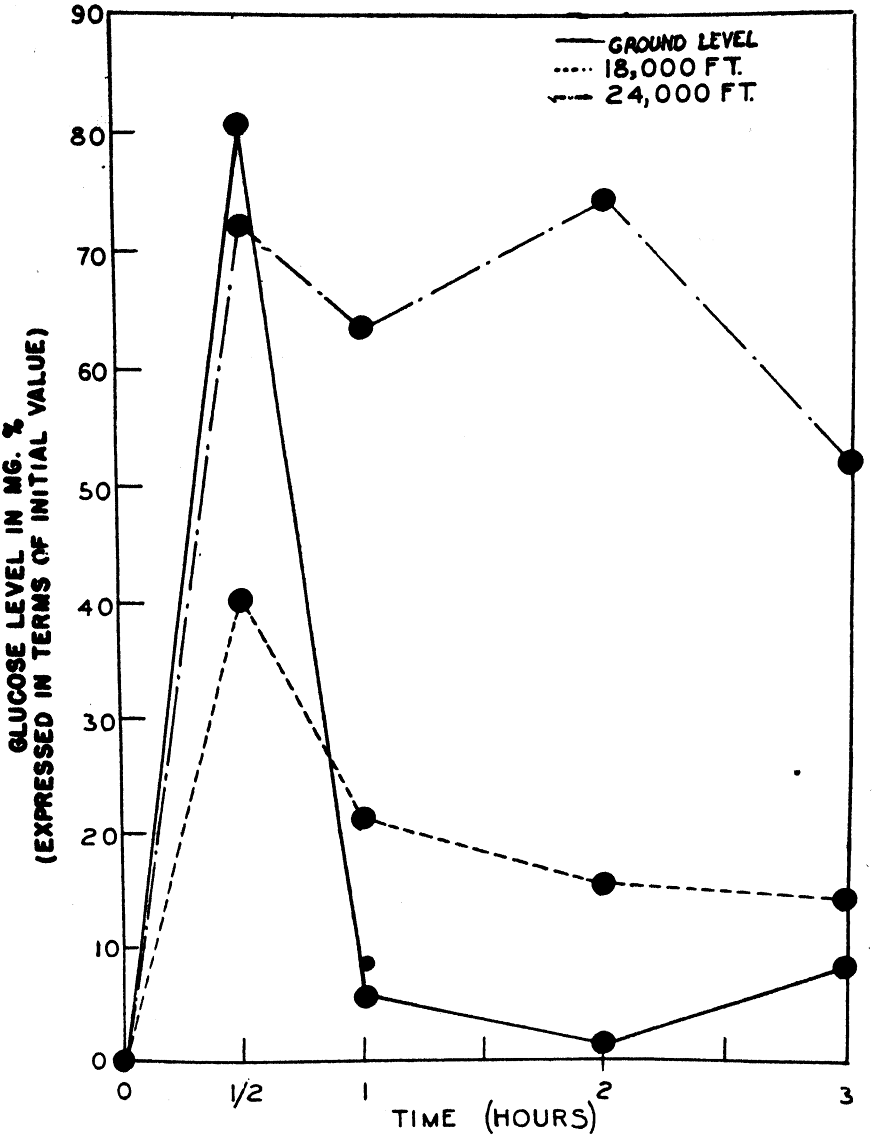

FIG. 1. MEAN DEXTROSE TOLERANCE CURVES on dogs 1, 2 and 3.

It is not surprising that the data presented in this paper differ somewhat from observations previously reported (2, 8, 16, 3) since none of these other experiments was performed under strictly comparable conditions. Acclimatization to altitude or prolonged exposure previous to the tolerance studies might well lead to marked variations in response to these tests. In view of the observations of Middlesworth, Kline and Britton (20) with respect to blood sugar levels rising, falling or remaining unchanged under anoxic conditions depending upon absorptive condition of the animal, length and degree of anoxic exposure and degree of acclimatization, it would seem logical to assume that these factors would be important in determining the response to glucose and insulin tolerance tests.

TABLE 2. INSULIN TOLERANCE TESTS

ALTITUDE	TIME	BLOOD GLUCOSE LEVEL (MGS. PER 100 CC.)			BLOOD GLUCOSE LEVEL (EXPRESSED AS ABSOLUTE CHANGE FROM INITIAL FASTING LEVEL,			
		Dog 1	Dog 2	Dog 3	Dog 1	Dog 2	Dog 3	Average
Ground level	0	126	106	113	0	0	0	0
	20 min.	92	71	76	−34	−35	−37	−35
	30 min.	97	61	90	−29	−45	−23	−32
	45 min.	84	80	88	−42	−26	−25	−31
	1 hr.	108	87	133	−18	−19	+21	−5
	1½ hrs.	126	82	131	0	−24	+19	−2
	2 hrs.	142	99	131	+16	−7	+19	+9
	45 min.	149	122	136	+23	+16	+24	+21
	60 min.	156	125	—	+30	+19	—	+25
18,000 ft.	0	125	112	126	0	0	0	0
	20 min.	105	87	106	−20	−25	−20	−22
	30 min.	104	97	118	−21	−15	−8	−15
	45 min.	100	90	106	−25	−22	−20	−22
	1 hr.	136	117	108	+11	+5	−18	−1
	1½ hrs.	123	117	122	−2	+5	−4	0
	2 hrs.	123	114	122	−2	+2	−4	−1
	45 min.	138	120	126	+13	+8	0	+7
	60 min.	127	111	122	+2	−1	−4	−1
24,000 ft.	0	134	102	96	0	0	0	0
	20 min.	98	76	84	−36	−26	−12	−25
	30 min.	138	86	77	+4	−16	−19	−10
	45 min.	132	92	95	−2	−10	−1	−4
	1 hr.	132	112	—	−2	+10	—	+4
	1½ hrs.	127	116	111	−7	+14	+15	+7
	2 hrs.	171	126	105	+37	+24	+9	+23
	45 min.	143	128	112	+9	+26	+16	+17
	60 min.	158	120	120	+24	+18	+32	+25

It is not the purpose of this study to assign the alterations observed in these tests to any single metabolic or hormonal factor. Several factors may be of importance in bringing about these changes. Although the relative importance of the rôles played by these various factors cannot be stated at the present time, the following must be considered as possible etiologic factors: 1) insufficient oxygen available to supply the energy necessary for the proper functioning of the enzymatic systems involved in carbohydrate metabolism; 2) increased activity of the sympathico-adrenal system; and 3) increased adrenal cortical activity. The relative importance of these various mechanisms is under investigation at the present time.

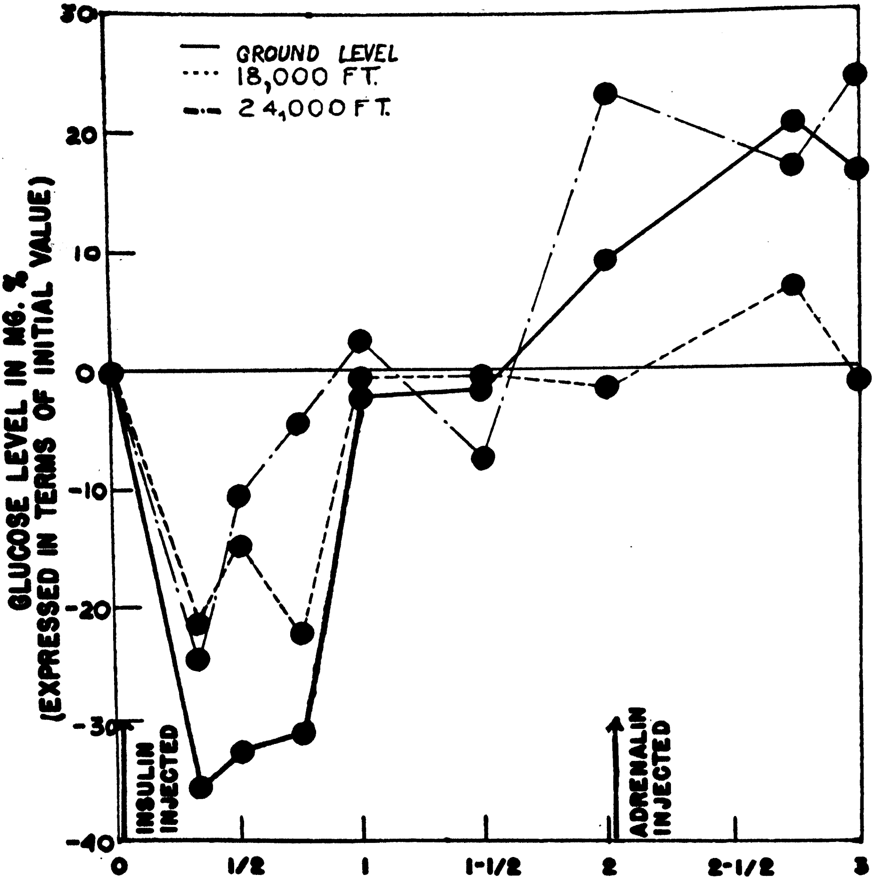

FIG. 2. MEAN INSULIN TOLERANCE CURVES on dogs 1, 2 and 3.

SUMMARY

Adult, male Dalmatian coach hounds were subjected to standard dextrose tolerance and insulin tolerance tests at ground level and at simulated altitudes of 18,000 feet and 24,000 feet. Differences in the responses to these tests were noted under the conditions of reduced barometric pressure.

The tolerance of the animals for intravenously administered dextrose was affected adversely by exposure to simulated altitude. This was manifested by a prolonged elevation of the blood sugar levels and by their failure to return to normal fasting values in a three-hour period. This effect was much more pronounced at 24,000 feet than at 18,000 feet.

The insulin tolerance tests revealed a decreased insulin response in the animals exposed to simulated altitudes with no appreciable change in the time required for the return of the blood glucose level to the initial fasting level. The response to intramuscularly administered adrenalin was not as marked at the simulated altitudes as at ground level.

REFERENCES

1. Baicenko, I. P. and A. N. Krestownikoff. Arbeitsphysiol. **6:** 359, 1932.
2. Bryan, A. H. and H. T. Ricketts. J. Clin. Endocrin. **4:** 450, 1944.
3. Clinton, M., Jr. and G. W. Thorn. War Medicine **4:** 363, 1943.
4. D'Angelo, S. A. This Journal **145:** 365, 1945.
5. Evans, G. This Journal **110:** 273, 1934.
6. Feldman, J., R. Cortell and E. Gellhorn. This Journal **131:** 281, 1940.
7. Ferraloro, G. Arch. Sci. Biol. (Italy) **13:** 109, 1929.
8. Forbes, W. H. This Journal **116:** 309, 1936.
9. Fraser, R., F. Albright. and P. H. Smith. J. Clin Endocrin. **1:** 297, 1941.
10. Glickman, N. and E. Gellhorn. This Journal **121:** 358, 1938.
11. Goldberger, S. Boll. Soc. ital. sper. **4:** 710, 1929.
12. Horvath, S. M. and C. A. Knehr. J. Biol. Chem. **140:** 869, 1941.
13. Johlin, J. M. and F. B. Moreland. J. Biol. Chem. **103:** 107, 1933.
14. Kellaway, C. H. J. Physiol. **53:** 211, 1919.
15. Langley, L. L. and R. W. Clarke. Yale J. Biol. & Med. **14:** 529, 1942.
16. Lewis, R. A., G. W. Thorn, G. F. Koepf and S. S. Dorrance. J. Clin. Invest. **21:** 33, 1942.
17. Madon, F. Arch. Sci. Biol. (Italy) **17:** 41, 1933.
18. McQuarrie, I., M. R. Ziegler, W. E. Stone, O. H. Wangensteen and C. Dennis. Proc. Soc. Exp. Biol. and Med. **42:** 513, 1939.
19. McQuarrie, I. and M. R. Ziegler. Proc. Soc. Exp. Biol. and Med. **39:** 525, 1938.
20. Middlesworth, L., R. F. van Kline and S. W. Britton. This Journal **140:** 474, 1944.
21. Sundstroem, S. S. and G. Michaels. The Adrenal Cortex in Adaptation to Altitude, Climate, and Cancer. Memoirs of the University of California. Vol. 12. P. 76.

HISTOCHEMICAL STUDY OF 'ALKALINE' PHOSPHATASE OF THE KIDNEY OF THE CASTRATED MOUSE AFTER STIMULATION WITH VARIOUS ANDROGENS[1]

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In previous reports it has been demonstrated that testosterone propionate (2) and many related steroids (3) produce a decrease in the 'alkaline' phosphatase of the kidney of the mouse. This report is an extension of these studies to several other androgens and a determination of the sites at which these changes occur as determined by the histochemical technic (4).

PROCEDURE

Male mice[2] of the Murray-Little dba strain were castrated under ether anesthesia at 16.0–19.5 grams body weight; one month later pellets of the various steroids[3] were implanted subcutaneously (5, 6). The amount of steroid absorbed was increased by implanting two or more pellets and decreased by pellets composed of mixtures of the steroid hormone and cholesterol in the desired proportions (6, 7).

The food, Purina fox chow checkers (table 2) or Rockland checkers (table 1), was removed from the mouse cages 18 to 20 hours before autopsy and the mice were killed by decapitation. The kidneys were removed, a 2 mm. slice was cut transversely through the mid-section of one of the kidneys, weighed and placed in 15 ml. of cold redistilled acetone for histo-'alkaline' phosphatase determination (4, 8). The remainder was weighed, homogenized and the enzymes determined as previously described (3).

RESULTS

In vitro studies (table 1). Androstenedione during only 10 days of treatment was very effective in decreasing the 'alkaline' phosphatase of the kidney. Dehydroiso-androsterone and iso-androsterone, even though absorbed at a relatively high dose level, caused only a small increase in kidney size which was accompanied by a small increase in the enzyme. Androsterone produced a small and androstanedione a moderate decrease in the amount of enzyme per gram of tissue. Neither of these latter two compounds, however, was as effective as

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[1] This investigation was aided by grants from the Josiah Macy Jr. Foundation. Parts of these data have been reported in the Josiah Macy Jr. Foundation Conference on Metabolic Aspects of Convalescence, fifth meeting, 136, and a review (1).

[2] The mice were provided by Dr. S. B. Warner of the Biological Station, Springville New York.

[3] The steroids were provided by Ciba Pharmaceutical Products, Inc.

androstenedione which produced only a slightly greater increase in kidney size but a much greater decrease in enzyme activity.

17-methylandrostanol-17α, one-3 produced an effect on the enzyme similar to that previously observed (3) when very potent renotrophic steroids, e.g., 17-methyl-testosterone, testosterone, etc. (cf. table 2) were used. At the lowest dose level, this compound produced an effect on the kidney weight and enzyme comparable to that obtained with androsterone.

The 'acid' phosphatase always increased (cf. 3) in amount at a rate equal to or slightly greater than the increase in kidney size (table 1).

TABLE 1. EFFECT OF STEROID HORMONES ON THE PHOSPHATASES OF THE KIDNEY OF THE CASTRATED MOUSE

TREATMENT	NO. MICE	NO. PELLETS	STEROID ABSORBED	KIDNEY WGT.	PHOSPHATASES			
					Alkaline		Acid	
					Total per cent	Per gram per cent	Total per cent	Per gram per cent
10 days								
			mgm.	*mgm.*				
Δ^4-Androstenedione-3,17	5	2	6.8	364	−39	−52	+21	−2
30 days								
Dehydroisoandrosterone	4	4	35.7	314	+16	+6	+36	+16
Isoandrosterone	4	4	28.2	330	+30	+12	+36	+15
Androsterone	4	4	13.0	348	+4	−13	+29	+4
Androstanedione-3,17	3	4	32.3	343	−15	−28	+14	−1
17-Methyl-androstanol-17α, one-3	3	1:4[2]	0.09	341	0	−15	+11	−7
	4	1:1[2]	0.45	476	−16	−49	+93	+14
	4	1	1.6	547	−40	−68	+97	+2
	4	2	3.3	566	−36	−68	+118	+9

[1] Change from the average values of 4 castrated control mice: Kidney weight = 284 grams; 'alkaline' phosphatase, total units = 116, units/gram = 401; 'acid' phosphatase, total units = 28, units/gram = 9.9 (cf. 3).

[2] Steroid: cholesterol composition of pellet.

Histochemical studies.[4] The 'alkaline' phosphatase (black areas, fig. 1, 2, 3 and 4) was present in the proximal convoluted tubule, the cells lining Bowman's capsule and at the turn of Henle's loop. It was specially dense along the brush border (cf. 4). After castration, the cells in the proximal convoluted tubule became smaller (cf. 9) and the enzyme more concentrated (fig. 1 and 2). As the kidney increased in size under steroid stimulation these cells became larger and the enzyme began to disappear in a definite pattern. The decrease was

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[4] Charles Luttrell assisted in the determination and study of the experiments in table 1 and Dr. Victor Emmel assisted in the study of the experiments in table 2.

TABLE 2. TREATMENT AND WEIGHT OF MOUSE KIDNEY STUDIED FOR 'ALKALINE' PHOSPHATASE BY THE HISTOCHEMICAL TECHNIC[1]

TREATMENT	NO. MICE	STEROID ABSORBED	KIDNEY
		mgm.	*mgm.*
Castrated	8		264
Normal	12		414
Testosterone	4	0.14	389
Testosterone	4	0.37	417
Testosterone	4	1.15	505
Testosterone	7	4.06	469
Testosterone	1	8.5	477
Testosterone	3	16.2	541
Testosterone propionate[2]	4	0.20	392
Testosterone propionate[2]	4	0.88	458
Testosterone propionate[2]	5	2.33	490
Testosterone propionate[2]	1	4.10	446
17-methyl testosterone[3]	4	0.14	415
17-methyl testosterone[3]	4	0.57	424
17-methyl testosterone[3]	4	1.30	466
17-methyl testosterone[3]	6	4.26	507
17-methyl testosterone[3]	1	8.10	540
17-methyl testosterone[3]	4	14.40	525
17-ethyl testosterone[4]	2	1.75	257
Androstanol-17α, one-3	5	0.65	424
Androstanol-17α, one-3	2	6.4	518
Androstanol-17α, one-3	4	7.1	505
Δ^4-Androstenedione-3, 17	2	19.7	420
Androstanediol-3α, 17α	3	0.25	383
Androstanediol-3α, 17α	1	1.5	470
Androstanediol-3α, 17α	4	3.8	472
Androstanediol-3α, 17α	4	5.5	464
Androstanediol-3α, 17α	3	5.9	466
Androstanediol-3α, 17α, acetate-3	6	2.3	421
Androstanediol-3α, 17α, acetate-3	8	3.0	399
Androstanediol-3β, 17α	4	2.0	333

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[1] The results obtained by *in vitro* homogenate studies have been reported (3).

[2] Perandren.

[3] Metandren.

[4] Pregnenynolone, lutocylol.

TABLE 2—*Continued*

TREATMENT	NO. MICE	STEROID ABSORBED	KIDNEY
		mgm.	*mgm.*
17-methyl androstanediol-3α, 17α	4	0.6	459
17-methyl androstanediol-3α, 17α	5	3.2	517
17-methyl androstanediol-3α, 17α	6	5.5	448
17-methyl androstanediol-3α, 17α	5	6.9	507
17-methyl androstanediol-3β, 17α	7	1.8	420
17-methyl Δ^5-androstenediol-3β, 17α	4	2.4	347
Δ^5-pregnenol-3β, one-20	1	4.6	305

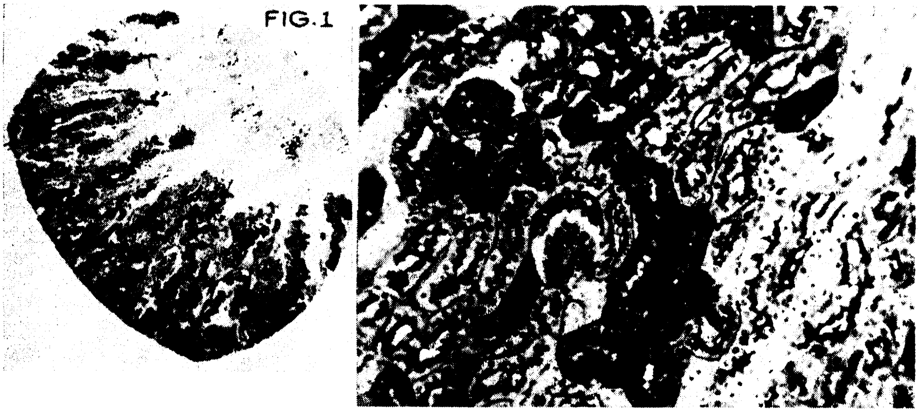

FIG.1

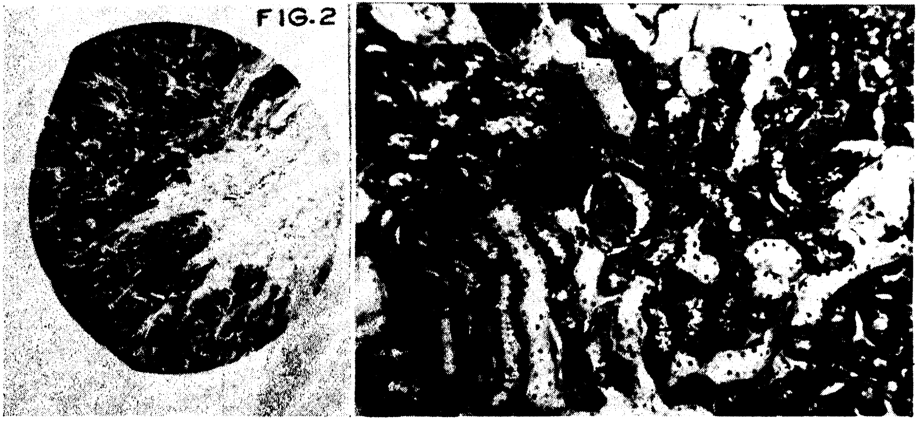

FIG.2

FIG. 1. KIDNEY OF A NORMAL MOUSE. Kidney weight = 419 mgm.; 'alkaline' phosphatase = 195 units/gram; A = 25×, B = 200×.

FIG. 2. KIDNEY OF A CASTRATED MOUSE. Kidney weight = 280 mgm.; 'alkaline' phosphatase = 414 units/gram; A = 25×, B = 200×.

progressive from the distal to glomerular end of the proximal convoluted tubule so that at maximal decrease in enzyme activity tubules of varying degrees of depletion were evident, e.g., testosterone (fig. 3) and 17-methylandrostanediol-3α, 17α (fig. 4). Furthermore, with depletion of enzymes from the distal end of the proximal convoluted tubule, the enzyme became slightly more concentrated at the glomerular end. The ability of the steroids to produce these effects paralleled their renotrophic activities.

FIG. 3

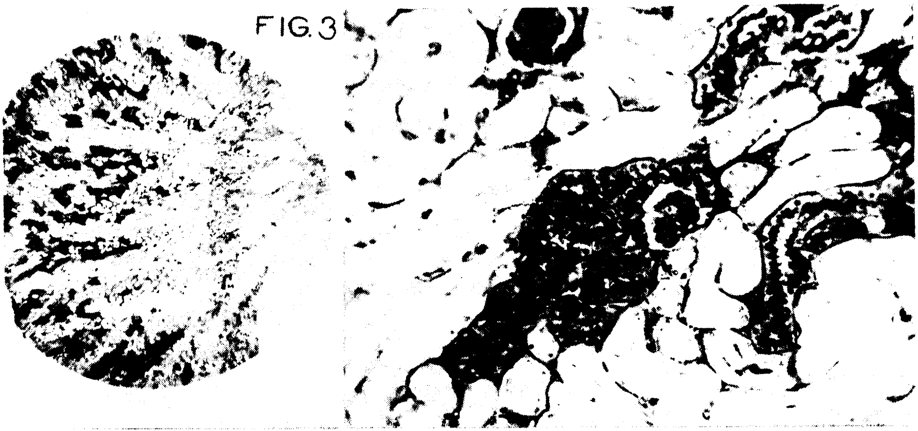

FIG. 4

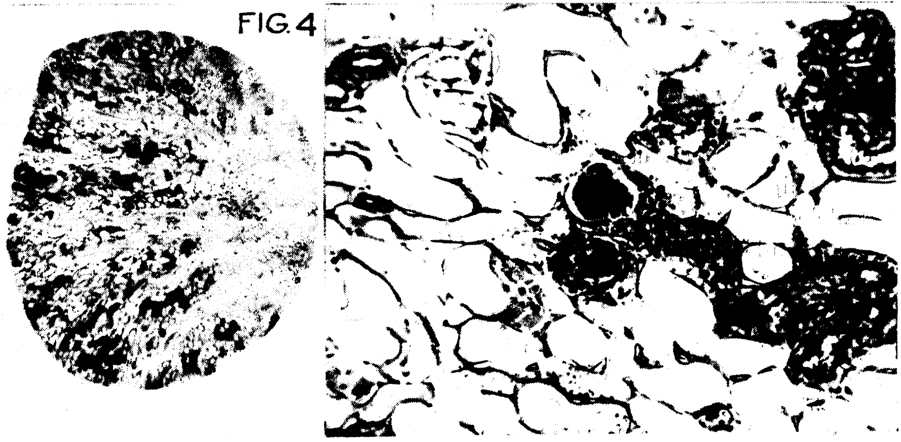

Fig. 3. Kidney of a castrated mouse implanted for 30 days with a pellet of 1:1 testosterone and cholesterol. Kidney weight = 477 mgm.; 'alkaline' phosphatase = 52 units/gram; A = 25×, B = 200×.

Fig. 4. Kidney of a castrated mouse implanted for 30 days with 4 pellets of 17-methylandrostanediol-3α, 17α. Kidney weight = 482 mgm.; 'alkaline' phosphatase = 63 units/gram; A = 25×, B = 200×.

The duodenum also was studied[5] but no noteworthy changes were observed.

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[5] A report on the observation of 'alkaline' phosphatase in the Golgi apparatus has been made (10).

DISCUSSION

The results obtained with the use of the steroids of this study not only confirm the previous results (1, 2, 3) but also indicate that in the very early stages of the increase in kidney size, there is a slight increase in the 'alkaline' phosphatase. When, however, the androgens stimulate the kidney weight to greater than about 20 per cent, there is a progressive decrease in the enzyme which parallels the increase in kidney size. Furthermore, the initial increase in the 'alkaline' phosphatase is in agreement with the hitherto apparently anomalous small increases of this enzyme noted in the kidneys of rats (8, unpublished), guinea pig (unpublished) and hamster (unpublished) for in these animals the androgens produce less than a 20 per cent increase in kidney size.

The initial increase in the 'alkaline' phosphatase followed by a progressive decrease from the distal end of the proximal convoluted tubule toward the glomerulus with an increase in concentration near the glomerulus suggests that, if the enzyme is involved in the reabosrption of certain materials (11), then these substances are not only filtered through the glomerulus in smaller and smaller quantities but also that they are being reabsorbed as rapidly as possible under androgen stimulation for presumably anabolic processes (12).

SUMMARY

The distribution of the 'alkaline' phosphatase of the kidney of the mouse has been studied after treatment with 18 different steroids many of which were studied at several dose levels. There was a progressive decrease in the enzyme from the distal end of the proximal convoluted tubule toward the glomerulus with a slight increase in concentration at the glomerular end. These changes paralleled the increase in kidney size under steroid stimulation so that at maximum stimulation the kidney showed varying degrees of depletion of the enzyme in its nephrons.

REFERENCES

1. Kochakian, C. D. In G. Pincus. Recent progress in hormone research, New York. **1:** 277, 1947.
2. Kochakian, C. D. and R. P. Fox. J. Biol. Chem. **153:** 669, 1944.
3. Kochakian, C. D. This Journal **145:** 118, 1945.
4. Goimori, G. J. Cell and Comp. Physiol. **17:** 71, 1941.
5. Kochakian, C. D. This Journal **142:** 315, 1944.
6. Kochakian, C. D. This Journal **145:** 549, 1946.
7. Fuenzolida, F. Rev. can. biol. **3:** 366, 1944; Rev. med. y. alimentacion **6:** 30, 1943–1944.
8. Vail, V. N. and C. D. Kochakian. This Journal **150:** 580, 1947.
9. Selye, H. J. Urology **42:** 637, 1939.
10. Emmel, V. M. Anat. Rec. **91:** 39, 1945.
11. Lundsgaard, E. Biochem. Ztschr. **264:** 209, 1933.
12. Kochakian, C. D. In Harris, R. S. and K. V. Thimann, Vitamins and Hormones, New York. **4:** 255, 1946.

VITAMIN A, IODIDE AND THYROTROPIC HORMONE CONTENT OF THE ANTERIOR PITUITARY

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Excessive intake of vitamin A reduces the secretion of thyrotropic hormone and also reduces the size of the thyroid and the metabolic rate (1–7). It is curious that excess vitamin A not only reduces the thyroid size but it also reduces the metabolic rate of hyperthyroid rats (7). If the reduction of the metabolic rate were brought about by thyroxine destruction, the lowered thyroxine level should increase the thyrotropic hormone secretion and thereby increase the size of the thyroid; but the size of the thyroid has not increased but decreased. A possible explanation of this unexpected effect is that vitamin A, by virtue of its double bonds, combines with the iodine of the thyroxine and this vitamin-iodine compound then behaves, in vitamin-antivitamin fashion, as thyroxine would in depressing thyrotropic hormone secretion. As the combination was expected to be between vitamin A and iodine of the thyroxine, a similar effect might be expected to be produced by feeding iodide together with vitamin A, since iodide given orally is said to be oxidized in the body to iodine unless such reducing agents as goitrogens are administered simultaneously (7a).

In iodine deficiency the thyroxine synthesis is decreased and this resultant low thyroxine level stimulates the secretion of thyrotropic hormone, which enlarges the thyroid gland. Iodide fed to animals increases the protein-bound iodine in the pituitary, which acts as thyroid hormone in inhibiting the formation of the thyrotropic hormone. This decrease in thyrotropic hormone leads to decreased activity of thyroid and lowers the basal metabolic rate (8). This paper reports a study of the effect of feeding excess vitamin A together with iodide on the thyrotropic hormone content in rats.

EXPERIMENTAL

Experiments were conducted with four groups of male albino rats; nine rats were placed in each group. One group served as control; the second group was fed 30,000 I. U. vitamin A daily in the form of percomorph oil; the third group was fed one gram potassium iodide daily; the fourth group was fed the above amounts of both vitamin A and potassium iodide. After 18 days on these diets, the rats were sacrificed and their thyroids, adrenals and pituitaries weighed in a precision balance. The results are shown in table 1.

The pituitaries were assayed for thyrotropic hormone on day-old chicks. Water suspensions of pituitaries were prepared by rubbing the gland in an agate mortar and made up to volume with distilled water, so that there were 10 mgm. of fresh pituitary tissue in one ml. of suspension. Five groups of day-old chicks were used; one group served as control while the other four groups were injected with the pituitary suspension of the above four groups of experimental rats for a period of four days. The chicks were sacrificed on the fifth day of the experiment and their thyroids dissected out and weighed. The results are shown in table 2.

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The chick thyroids, prepared histologically, were stained with hematoxylin and eosion. The diameters of the epithelia of the thyroid acini were measured and the results are presented in table 2.

TABLE 1. EFFECT OF POTASSIUM IODIDE AND VITAMIN A ON THE ENDOCRINE ORGANS OF THE RAT

GROUP	NO. OF RATS	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	GAIN IN WEIGHT PER DAY	THYROID WT. PER 100 GRAMS BODY WT.	ADRENAL WT. PER 100 GRAMS BODY WT.	PITUITARY WT. PER 100 GRAMS BODY WT.
		grams	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*
Control	9	89	167	4.3	6.56	11.89	3.46
Fed one gram KI daily	9	89	150	3.4	5.89	11.74	3.69
Fed 30,000 I.U. vitamin A daily	9	93	139	2.6	4.74	16.70	4.22
Fed vitamin A (30,000 I.U.) and KI (1 gram) daily	9[1]	86	112	1.4	6.61	18.44	4.78

[1] Three of the rats died during the experiment.

TABLE 2. ASSAY OF THYROTROPIC HORMONE BY CHICK AND HISTOLOGICAL METHODS (4 mgm. pituitary tissue injected)

GROUP	NO. OF CHICKS	BODY WEIGHT	THYROID WT. PER 100 GRAMS BODY WT.	HEIGHT OF EPITHELIA
		grams	*mgm.*	*micra*
Control	11	47	5.99	3.78
Control rat pituitary group	11	47	11.23	5.88
KI-fed rat pituitary group	11	48	10.91	—
Vitamin A-fed rat pituitary group	11	51	9.82	5.04
Vitamin A- and KI- fed rat pituitary	7	47	11.83	5.78

DISCUSSION

Table 1 indicates that on the high vitamin A diet the daily gain in body weight is 2.6 grams as contrasted to 4.3 grams in the control group and that the thyroid weight on the high vitamin A diet is significantly lower and the adrenal weight significantly higher than on the control diet. The pituitary weight also increased, but the iodide-fed group shows a smaller thyroid weight than the control group. This is probably due to the fact that iodide after *in vivo* oxidation to iodine causes iodination of anterior pituitary tissue protein (8) or of proteins elsewhere, leading to the formation of thyroxine, which depresses the formation of thyrotropic hormone by the anterior pituitary, and consequent decrease in thyroid size. There is no change in the size of adrenal or pituitary. When both vitamin A and potassium iodide are fed, the combination becomes rather toxic; three of the nine rats died during the experimental period. Toxicity was not due, however, to iodism, as the same amount of iodide fed to other rats did not produce toxic symptoms. The toxic condition is also shown by the increase in adrenal size, with no concomitant change in thyroid size.

Both iodide and vitamin A have been found to lower the thyrotropic hormone secretion (table 2). The metabolic rate-lowering effect of vitamin A in normal rats can be explained by the thyrotropic-depressing effect of the assumed formation of iodized vitamin A, as previously explained, but not in rats made hyperthyroid by injecting thyroxine. Therefore, vitamin A must act in the peripheral end organs by preventing the action of thyroxine on the enzyme systems on which thyroxine acts. As vitamin A lowers the thyrotropic hormone content of the pituitary (table 2), it was assumed that the effect must be produced by the vitamin A-thyroxine compound which acts as thyroxine on the anterior pituitary. There is no direct evidence that such a compound is formed in the body. *In vitro*, however, there is spectroscopic evidence for such a combination with carotene and thyroxine (9, 10). As vitamin A retains most of the chemical properties and also the lyophobic characteristics of its parent chemical substance, carotene, it is expected that, like carotene, it will be iodinated at some of its double bonds. This may be made possible by feeding iodide when the iodide is oxidized into elementary iodine in the body. The toxicity of vitamin A in combination with iodide feeding also shows that vitamin A combines with iodine liberated *in vivo* from iodides. Thyroxine-iodine may similarly combine with vitamin A. But with potassium iodide feeding and, therefore, with *in vivo* liberation of elementary iodine, since vitamin A does not lower thyrotropic hormone secretion by the anterior pituitary, it must be assumed that the effect on the pituitary is exercised by vitamin A alone and not by the vitamin A-thyroxine compound if such a compound is formed. It would appear that part of the vitamin A supplied neutralizes some of the metabolism-stimulating effect of thyroxine while the remaining of the vitamin supplied, which is not combined with thyroxine, acts on the pituitary and lowers the thyrotropic hormone secretion.

The results reported in this paper appear to be the specific effect of vitamin A and cannot be due simply to nonspecific toxicity as under toxic conditions; though the adrenal gland is enlarged, there is no evidence for a decrease in thyroid size. Again vitamin A depresses the hypermetabolism produced by injection of thyroxine, but not of dinitrophenols. Neither can the results be due to failure to gain body weight, for the difference between the groups is more than can be explained by a simple change in body weight. It will be noted in table 1 that as the body weight gain decreases progressively in the four groups of rats, thyroid weight does not correspondingly decrease, but in the last group, with the least body weight gain, the thyroid weight is comparable to that of the control rats.

The way in which vitamin A lowers thyrotropic hormone secretion (table 2) is not understood. It has been assumed that vitamin A may compete with thyroxine on the metabolic systems and thus lower the metabolic effect. But as there is no similarity between the chemical configurations of vitamin A and thyroxine, this assumption cannot be proved.

From these experiments it is concluded that vitamin A reduces the thyroid size by depressing the secretion of thyrotropic hormone by acting directly on the anterior pituitary and not by forming an iodide compound.

SUMMARY

1. Vitamin A in large doses (30,000 I. U. per day per rat) decreases the thyroid size. This effect is apparently produced by lowering the secretion of thyrotropic hormone of the anterior pituitary. Excess vitamin A also increases the size of the adrenal gland.

2. Potassium iodide slightly lowers the thyroid size and depresses the thyrotropic secretion, or inactivates the thyrotropic hormone.

3. Iodized vitamin A has no influence on thyroid size or on the thyrotropic secretion, confirming the conclusion that vitamin A lowers the thyrotropic secretion directly and not by compound formation with thyroxine.

4. Thyrotropic hormone has been assayed by the response in day-old chicks and confirmed histologically by measuring the height of epithelia in thyroid acini.

REFERENCES

1. McCarrison, R. Brit. Med. J. **1:** 989, 1931.
2. Mitzkewitsch, M. S. Arch. exptl. Path. und Pharmakol. **174:** 339, 1934.
3. Uotilia, U. Virchow's Arch. path. Anat. **301:** 535, 1938.
4. Schulze, E. and G. Hundhausen. Arch. exptl. Path. und Pharmakol. **192:** 43, 1939.
5. Drill, V. A. Physiol. Revs. **23:** 355, 1943.
6. de Visscher, M.: La Régulation Hormonale du Métabolisme et le Vitamin A. Paris: Masson et Cie., 1946.
7. Sadhu, D. P. and S. Brosy. This Journal **149:** 400, 1947.
7a. Albert, A. *et al.* Endocrinology **40:** 299, 303 and 361, 1947.
8. McClendon, J. F. and Wm. C. Foster. Federation Proc. **6:** 275, 1947.
9. Euler, H. and E. Klussman. Z. physiol. Chem. **213:** 21, 1932.
10. Fleischmann, W. and S. Kann. Wien. klin. Wochschr. **49:** 1488, 1936.

SHRINKAGE OF LYMPHATIC TISSUE IN RATS FOLLOWING INJECTIONS OF INSULIN

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It is well established that acute injury of the body initiates a chain of reactions by which the anterior pituitary stimulates the adrenal cortex to secrete a hormone which in turn causes dissolution of lymphocytes in lymphatic tissue, releasing protective substances, this constituting a defense reaction or adaptation syndrome. Consistent with the interesting hypotheses of Vogt (1) and of Long and Fry (2), it may be postulated that following acute bodily injuries, the prompt response of the sympathetic nervous system in releasing epinephrine from the medulla is followed by the slower but more sustained activity of the cortex—a coordinated process which gives meaning to the anatomic juxtaposition of cortex and medulla. In support of this concept, there may be gathered from the literature various data concerning separate phases of this series of reactions. It has been shown that following injections of epinephrine in animals, the cortex hypertrophies (3); adrenal cortical secretion occurs (1); and the cortex loses its lipoids (2), which is now known to be indicative of discharge of hormone. Selye (4, 5) has included epinephrine injections as one of the many damaging agents causing the 'alarm reaction' with resultant reduction in lymphatic tissue. However, adrenal demedullation does not prevent the fall in adrenal cholesterol after exposure to cold (6) and Long (7) states "the hypothesis that sympathetic stimulation (and release of epinephrine) is an essential link in the pituitary-adrenal cortex activation is at present not completely established." It seems of interest, therefore, to learn more about the factors which start this response.

Experiments were planned to see if repeated large doses of insulin, known to result in stimulation of the adrenal medulla through the sympathetic nervous system, would release enough endogenous epinephrine to reproduce the effect of injections of exogenous epinephrine on lymphatic tissue. It is known that repeated doses of insulin may cause enlargement of the adrenal cortex. The literature on this subject has been reviewed (8), but data were not found on whether or not insulin injections caused shrinkage of lymphatic tissue. Latta and Henderson (9) in 1937 found that repeated insulin injections into rats caused transient reduction in numbers of lymphocytes in the blood, and hyperplasia of reticular cells and lymphocytes in lymph nodes with degenerated cells in lymph nodes, which in the light of our present knowledge we would attribute to adrenal cortical activity. Weights of lymphatic tissues were not given and it was not suggested at the time that the destruction of lymphocytes might be, not a direct action of insulin, but a result of a response provoked by insulin in other organs.

EXPERIMENTS

A group of nine rats were injected repeatedly with epinephrine to establish data as a standard of comparison for the effect of insulin. They were injected two or three times daily with 1:1000 solution diluted so as to give individual doses varying from 0.010 mgm. to 0.070 mgm. per 100 grams body weight, over a period of three to seven days. Controls were nine rats either litter-mates of the same sex or rats of equal body weight, either uninjected or injected with water to control the effect of trauma.

Another group of 16 rats were given insulin injections, usually in ascending doses, twice daily, varying from 0.2 to 2.5 units per 100 grams body weight over periods of three to seven days, in order to cause repeated severe hypoglycemia. They were not fasted except in some cases for two hours after the injection, but were allowed food so as to avoid the effect of inanition on the adrenal cortex and lymphatic tissue. Controls were 16 rats, either litter-mates or rats of the same body weight and same sex and they were either not injected or injected with water. With ascending doses some of the rats were able to survive very large doses, presumably as a result of adrenal cortical hypertrophy. This may be the explanation of the increased tolerance of the rabbit to huge doses of insulin described in previous experiments (10). When necessary, glucose was given by mouth to relieve hypoglycemia. Occasionally it was necessary to inject glucose intraperitoneally to save the life of the rat, and then the control rat was similarly injected. At appropriate intervals the rats were either killed with anesthetic or allowed to die of hypoglycemia. Tissues were weighed rapidly to the nearest mgm. Some injected rats lost weight, so the final body weights varied slightly from the controls.

As rats of different ages and sizes were used, there is great variation in the weights of the different organs (table 1). Each injected rat was paired with a rat of similar body weight, and the purpose was not to compare absolute weights but to determine in the case of each pair whether or not the injected rat showed alteration from the control. These alterations are expressed as percentage increase or decrease in table 1 and figure 1. It may be seen that with few exceptions the lymphatic tissue shrinks as the result of the injections and the adrenals enlarge. Since much evidence in the literature indicates that adrenal hypertrophy and secretion is always accomplished by way of the pituitary, the insulin and epinephrine injected were presumably effective in stimulating the pituitary. In the instances in which adrenal weights were decreased, probably the dosage had been so intense as to lead toward adrenal exhaustion with consequent lessened shrinkage of lymphatic tissue.

An attempt was made to see if adrenalectomy would abolish the effect of insulin on lymphatic tissue. Adrenalectomized rats were kept in good condition by NaCl in the drinking water. Two weeks or longer after operation they were injected with repeated doses of insulin. Difficulties were encountered because of the well known fact that adrenalectomized animals are extremely sensitive to insulin. Many died in hypoglycemia soon after receiving minute doses of

TABLE 1. COMPARISON OF INJECTED RATS WITH CONTROLS

	NO. OF RATS	FINAL BODY WEIGHT, MEAN	THYMUS, MEAN	MESENTERIC LYMPH NODES, MEAN	ADRENALS, MEAN	PERCENTAGE CHANGE IN WEIGHT OF INJECTED RATS AS COMPARED WITH CONTROLS		
						Thymus, mean	Mesenteric lymph nodes, mean	Adrenals, mean
		gms.	*gms.*	*gms.*	*gms.*	*per cent*	*per cent*	*per cent*
Controls	9	248	0.35 ±0.059[1]	0.365 ±0.044[1]	0.039 ±0.003[1]	−39%	−13%	+9%
Injected with epinephrine	9	241	0.234 ±0.054	0.306 ±0.031	0.043 ±0.003			
Controls	16	203	0.354 ±0.028	0.314 ±0.007	0.038 ±0.003	−13%	−19%	+8%
Injected with insulin	16	192	0.306 ±0.026	0.244 ±0.013	0.054 ±0.003			

[1] Standard error.

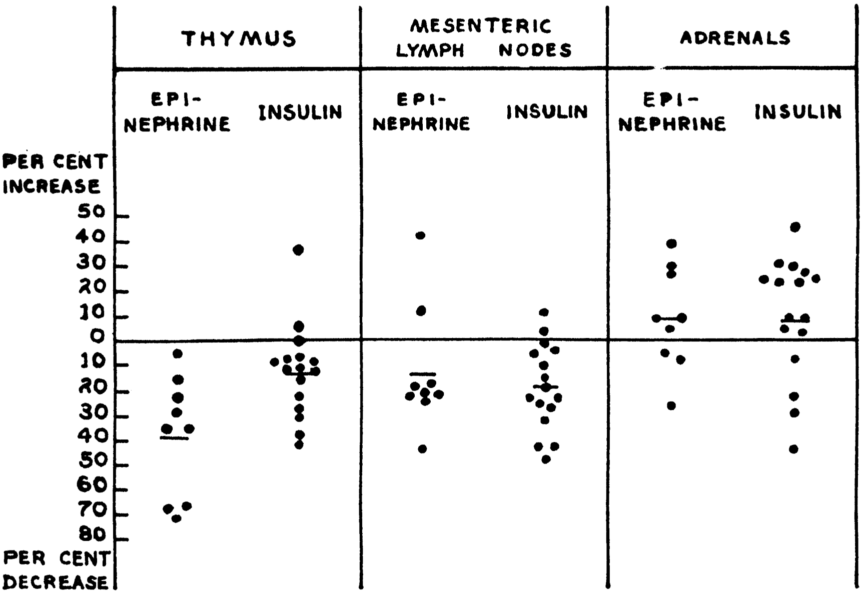

FIG. 1. PERCENTAGE CHANGE IN WEIGHTS of organs of rats injected with epinephrine and with insulin in comparison with controls.

insulin such as 0.025 units per 100 grams body weight. It seemed impossible to maintain hyoglycemia by any dose of insulin for periods comparable to those in the series of intact rats. The weights of lymphatic tissue were so variable that no conclusions could be drawn. Attempts at denervating the adrenal medulla and leaving the cortex intact resulted in so much scarring in the region of the adrenal that the results were not thought to be valid. It was therefore not possible to prove the rôle of the adrenal medulla or cortex in these experiments.

SUMMARY

The experiments give evidence that, in rats, exposure to repeated injections of insulin causes reduction in weight of lymphatic tissue. Since insulin hypoglycemia is known to result in discharge of endogenous adrenalin, it is possible that secretion of the adrenal medulla has activated the adrenal cortical-lymphatic tissue reaction.

REFERENCES

1. Vogt, M. J. Physiol. **103:** 317, 1944.
2. Long, C. N. H. and E. G. Fry. Proc. Soc. Exper. Biol. and Med. **59:** 67, 1945.
3. Vogt, M. J. Physiol. **104:** 60, 1945.
4. Selye, H. Endocrinology **21:** 165, 1937.
5. Selye, H. J. Clinical Endoc. **6:** 117, 1946.
6. Long, C. N. H. Proc. Johns Hopkins Med. Soc., Bull. Johns Hopkins Hosp. **78:** 317, 1946.
7. Long, C. N. H. Proceedings of the Laurentian Hormone Conference, Vol. 1. Academic Press Incorp., N. Y. 1947.
8. Tepperman, J., F. L. Engel and C. N. H. Long. Endocrinology **32:** 373, 1943.
9. Latta, J. S. and J. W. Henderson. Folia Hematol. **57:** 206, 1937.
10. Zeckwer, I. T. This Journal **106:** 273, 1933.

INTERRELATIONSHIPS OF SPERMATOZOA COUNT, HYALURONIDASE TITER AND FERTILIZATION

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The problem of fertilization in animals and man has engaged the attention of a number of workers and the knowledge accumulated has brought into focus three factors which appear to be of significance in the process of fertilization. These are the preparation of the ovum prior to sperm penetration, the influence of spermatozoa numbers and the relation of the enzyme hyaluronidase to fertilization.

The cumulus cells of mammalian ova have been described as being cemented together by a viscous substance (1). It has been suggested that this cementing substance is hyaluronic acid and that it is rendered non-viscous by the action of hyaluronidase present in normal semen, thus allowing dispersal of the cumulus cells in the process of fertilization (2, 3). The amount of hyaluronidase present in semen has been reported as varying positively in a linear fashion with the number of spermatozoa (4, 5).

Many attempts have been made to determine the minimal and optimal sperm concentrations required for fertilization in various species (6, 7, 8) but, in general, there has been little agreement in comparable situations.

It is the purpose of this paper to report our studies on the quantitative interrelationships of the spermatozoa count, hyaluronidase titer and percentage fertilization in cattle and to indicate their probable importance to the problem of fertilization as a whole.

METHODS

Seminal specimens were collected from individual bulls and part of each specimen was set aside for spermatozoa count and hyaluronidase assay, which were made within twenty hours after collection. Specimens were diluted 1:55 with a sterile diluent consisting of equal parts of egg yolk and distilled water containing 36 grams of sodium citrate and 3 grams of sulfanilamide per liter. Diluted seminal specimens were kept cold during transit to 45 inseminators throughout Ohio. The majority of inseminations were accomplished within 48 hours of semen collection and the remainder within 72 hours. One cc. of diluted semen was used in each insemination.

The criterion for successful fertilization was the failure of the cattle owner to request another insemination (non-return). Since three services are guaranteed

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by the original fee, unsuccessful inseminations have invariably been followed by further requests (return). For any one cow, only the fertilization results of a maximum of two inseminations were recorded. Beyond this number the fertility of the cow and the assurance of another request for semen were open to question. A period of three months from the time of insemination was allowed to elapse before a non-return was considered valid.

Spermatozoa counts. To minimize the 'normal' counting chamber and pipette error, all spermatozoa counts were made on one Spencer 'bright-line' haemacytometer and with the same two red cell dilution pipettes. A diluting fluid containing 5 per cent sodium bicarbonate and 1 per cent phenol was used. In making a count the semen sample was thoroughly mixed and drawn to the 0.5 mark, resulting finally in a dilution of 1:200. The actual determination was made by filling the counting chamber and counting the four corner and center squares of the customary red cell area. Seven zeros were added to the total number of sperm in the five squares to give the count per cc.

Individual counts were repeated 15 to 20 times in an effort to determine the amount of variance between counts. It was found that all counts for a single specimen fell within ±18 per cent of the mean count. Werthessen *et al.* (4) arrived at a ±20 per cent range for sperm count variance. Magath *et al.* (9) found the error of a single estimate of the erythrocyte count to be ±16 per cent. Apparently, the greater variance with spermatozoa is due to some property of the semen, possibly its greater viscosity. All sperm counts made in the experimental work were an average of four determinations. Sperm counts made were total cell counts; motility and morphology studies could not be accomplished under the conditions of semen collection.

Hyaluronidase assay. Hyaluronidase assays were made turbidimetrically (5). This method is based on the observation that pure hyaluronate at pH 4.2 gives a stable colloidal suspension when mixed with dilute blood serum, whereas, hyaluronate depolymerized by hyaluronidase action remains clear. The hyaluronidase unit is designated as the turbidity-reducing unit (TRU) and is defined as the amount of hyaluronidase which will reduce the turbidity produced by 0.2 mgm. of hyaluronic acid under given conditions to the equivalent turbidity produced by 0.1 mgm. of hyaluronic acid in 30 minutes at 37°C. Turbidity measurements were made with an Evelyn photoelectric colorimeter using a 660 mu color filter. Colorimeter readings plotted against varying hyaluronidase concentrations produced an S-shaped curve but in the region between 0.2 mgm. and 0.1 mgm. the curve was almost a straight line. We have found that greater rectification of the linear function was obtained when turbidity readings were plotted against the logarithms of the hyaluronidase dilutions rather than the dilutions themselves. Therefore, within the test range of hyaluronic acid and in utilizing the above modification, decrease in turbidity is a linear function of enzyme concentration. All hyaluronidase values were calculated in this fashion.

It was found that when different batches of horse serum or the same batch at successive intervals of approximately a week, whether previously diluted or not, was used in the turbidimetric test procedure, a single semen specimen gave significantly different hyaluronidase titers. Upon further investigation it became apparent that the varying titers were associated with inequalities in the turbidity ranges between the 0.2 mgm. and the 0.1 mgm. standards caused by the serum-hyaluronate complex. Large turbidity ranges caused lower values and, conversely, small ranges gave increased values. This relationship, however, was not strictly linear and it did not prove feasible to obtain a correction factor for varying sera. To obtain comparable hyaluronidase values, therefore, only sera exhibiting turbidity ranges of 6.0 ± 1 Evelyn galvanometer scale divisions were used in obtaining the enzyme titers.

The enzyme substrate, pure sodium hyaluronate, was prepared from human umbilical cords according to the method of McClean (10) as modified by Rogers (11).

EXPERIMENTAL RESULTS

A total of 2992 inseminations were made in 2092 cows. The comparative success of first and second inseminations are given in table 1.

TABLE 1. Fertilization results with first and second inseminations

	TOTAL	1ST INSEMINATION	2ND INSEMINATION
Fertilizations	1618	1192	426
Non-fertilizations	1374	900	474
Per cent fertilizations		57.0	47.3
Per cent non-fertilizations		43.0	52.7
Inseminations	2992		
Cows	2092		

It will be seen from table 1 that 57 per cent of the cows under observation were successfully fertilized by the first insemination while 47.3 per cent of the remainder were fertilized by the second insemination. Both fertilization percentages lie within the usual range of successful fertilizations obtained by most insemination groups. In view of the fact that variations in the fertilizing capacities of different semen specimens and in appropriate insemination time and technique are normally operative, it can be concluded that only a relatively few cows can be suspected of sterility and that the animals used represented as a whole a statistically appropriate sampling for the purpose at hand.

The data in table 2 are arranged in ascending order of percentage fertilizations (C) which are based on the proportion of non-returns for the particular semen specimen. Spermatozoa counts (A) are given in millions of sperm per cc. while hyaluronidase activity (B) is measured in turbidity-reducing units per cc. There are a total of 49 individual semen specimens from 25 bulls for which data are listed.

The results of statistical analysis of the above data are given in table 3. It will be seen that the correlation coefficient between sperm count and hyaluronidase titer is +.27891, the t value for this coefficient being 1.991. Since the level

TABLE 2. SPERM COUNT, HYALURONIDASE TITER AND PERCENTAGE FERTILIZATION FOR INDIVIDUAL SEMEN SPECIMENS

SPERM COUNT IN MIL./CC. (*A*)	HYALURONIDASE TRU/CC. (*B*)	NON-RETURN	RETURN	TOTAL RETURN, NON-RETURN	PERCENTAGE FERTILIZATION (*C*)
560	2933	20	60	80	25.00
1350	3673	12	25	37	32.43
1310	2956	20	40	60	33.33
400	1978	7	12	19	36.84
1090	3378	6	10	16	37.50
600	4333	23	38	61	37.70
470	1173	19	29	48	39.58
1120	2160	37	55	92	40.22
940	2167	19	25	44	43.18
470	1400	36	46	82	43.90
540	2200	32	40	72	44.44
1520	1643	27	32	59	45.76
1560	1956	33	39	72	45.83
410	1341	37	39	76	48.68
1130	3200	28	29	57	49.12
540	1278	29	28	57	50.88
1780	4200	22	21	43	51.17
1220	2986	13	12	25	52.00
1660	2134	26	24	50	52.00
1080	1190	44	39	83	53.01
690	1613	12	10	22	54.55
1590	1422	52	43	95	54.74
1520	1991	11	9	20	55.00
570	1750	25	20	45	55.56
2440	2533	29	23	52	55.77
700	2867	50	37	87	57.47
1060	2107	34	25	59	57.63
860	2444	36	25	61	59.02
1200	1369	37	25	62	59.68
760	1567	6	4	10	60.00
490	1583	29	19	48	60.42
600	1213	28	18	46	60.87
770	1956	42	27	69	60.87
1020	1422	38	24	62	61.29
1150	1511	39	24	63	61.90
780	1417	43	26	69	62.32
710	831	43	26	69	62.32
1240	3289	50	30	80	62.50
1480	2883	43	25	68	63.24
1090	1700	40	23	63	63.49
1730	3209	41	23	64	64.06
2730	2850	38	21	59	64.41
1890	1333	50	27	77	64.94
1380	1200	65	34	99	65.66
1020	1894	49	25	74	66.22
1320	1389	47	22	69	68.12
1100	1400	15	6	21	71.43
1200	3100	33	13	46	71.74
940	2167	26	10	36	72.22
53780	104289	1618	1374	2992	2660.01

N = 49.

of significance $P = .05$ has a t value less than 1.991, it was concluded that r_{AB} is statistically significant. The same conclusion is reached for $r_{BC} = -.32290$ whose t value lies between the $P = .02$ and $P = .01$ levels of significance. However, the correlation coefficient of sperm count with fertility $r_{AC} = +.24436$ has a t value between the $P = .1$ and $P = .05$ levels and hence cannot be considered significant.

All equations derived in this paper have been adjusted for values obtained from undiluted semen specimens. For computational use with diluted semen, appropriate changes in constants must be made for each particular dilution. Three regression equations (I, II, III) are listed in table 3 from which corresponding values of hyaluronidase titer and percentage fertilization can be obtained by inserting in the equations the respective values of sperm count or hyaluronidase titer. Percentage fertilization values derived from the hyaluronidase substitution equation will have a greater reliability than those derived from the sperm count substitution equation due to the smaller standard error of estimate of the former and the fact that r_{AC} is not significant.

TABLE 3. ZERO ORDER REGRESSION EQUATIONS AND COEFFICIENTS OF CORRELATION

	ZERO ORDER REGRESSION EQUATIONS	STANDARD ERROR OF ESTIMATE
I	$B = 1617.36 + 465.57 \times 10^{-3}(A)$	$S_B = 806.14$
II	$C = 48.37 + 539.51 \times 10^{-5}(A)$	$S_C = 10.72$
III	$C = 63.34 - 425.45 \times 10^{-5}(B)$	$S_C = 10.46$
	CORRELATION COEFFICIENT	t VALUE
	$r_{AB} = +.27891$	1.991
	$r_{AC} = +.24436$	1.728
	$r_{BC} = -.32290$	2.339
	LEVEL OF SIGNIFICANCE	CORRESPONDING t VALUE
	$P = .05$	1.960
	$P = .02$	2.326
	$P = .01$	2.576
	$P = .001$	3.291

In deriving our statistical results thus far we have assumed an oversimplified condition. For instance, in obtaining the coefficient of correlation between sperm count and fertility we have assumed that no other influencing factors were present. Actually, we know that fertilization is also affected by hyaluronidase titer and in a negative fashion. Similarly, in determining the relationship between hyaluronidase and fertilization, the influence of sperm count must be taken into consideration. Thus, it is important to know the correlation between fertilization and sperm count when the influence of hyaluronidase titer is held constant and, similarly, the correlation between fertilization and hyaluronidase when sperm count is held constant. Fortunately, in being able to obtain the coefficient of net (partial) correlation, we possess a means of obtaining these ends. The coefficient of net correlation for sperm count and percentage fertilization when the influence of hyaluronidase titer is held constant ($r_{AC.B}$) turns out to be +.36730 with $t = 2.678$ (table 4). Correspondingly, $r_{BC.A} = -.41999$ with $t = 3.139$. Both of these coefficients are highly significant as their t values indicate.

In obtaining regression equations II and III for percentage fertilization we were guilty of incompleteness as well as over-simplification. It is desirable to obtain a regression equation for percentage fertilization which would take into account the fact that fertilization is a function of hyaluronidase titer and of sperm count, both variables acting upon it simultaneously and in opposite directions. This equation is obtained by deriving a multiple regression equation for percentage fertilization, with sperm count and hyaluronidase titer as the two independent variables:

TABLE 4. MULTIPLE REGRESSION AND NET CORRELATION RESULTS

COEFFICIENT OF NET CORRELATION	t VALUE
$r_{AC.B} = +.36730$	2.678
$r_{BC.A} = -.41999$	3.139
MULTIPLE REGRESSION EQUATIONS	
IV $C_1 = 799.08 \times 10^{-5}(A) - 557.54 \times 10^{-5}(B)$	
V $C = 57.38 + 799.08 \times 10^{-5}(A) - 557.54 \times 10^{-5}(B)$	
STANDARD ERROR OF ESTIMATE	
$S_{C.AB} = 9.73$	
COEFFICIENT OF MULTIPLE CORRELATION	t VALUE
$R_{C.AB} = +.43890$	3.313

$$C_1 = 799.08 \times 10^{-5}(A) - 557.54 \times 10^{-5}(B) \qquad \text{IV}$$
$$C = 57.382 + 799.08 \times 10^{-5}(A) - 557.54 \times 10^{-5}(B) \qquad \text{V}$$

where A is millions of sperm/cc. and B is hyaluronidase titer in TRU/cc.

Equation IV contains the net coefficients of estimation, indicating the relative simultaneous effect of sperm count and hyaluronidase titer on fertilization. Equation V is the actual predictive equation for percentage fertilization. The standard error of estimate for V ($S_{C.AB}$) is 9.73. A comparison of this figure with the values of $S_C = 10.720$ and $S_C = 10.463$ which were obtained with equations II and III denotes the increased reliability of values obtained with equation V.

Finally, the derivation of one more coefficient is highly instructive. The coefficient of multiple correlation $R_{C.AB}$ is an indicator of the proportion of the total variation in percentage fertilization which is being measured by the multiple regression equation. $R_{C.AB}$ turns out to be +.43890 with a t value of 3.313, indicating that this coefficient is highly significant statistically. The difference between the square of the multiple correlation coefficient ($R^2_{C.AB} = .22385$) and unity indicates the proportion of the total variation which is accounted for by factors other than sperm count and hyaluronidase titer. That these factors represent the major portion of the total variation is readily understandable in view of the fact that successful fertilization also depends upon insemination occurring during the short estrus period, the use of uniformly appropriate insemination techniques in the field and the physiological fitness of the individual cow for pregnancy during the first three months following conception.

DISCUSSION

With the demonstration that hyaluronidase disperses the cumulus cells of mammalian ova and the consequent implication of the enzyme in the process of fertilization, the hypothesis has been advanced that millions of spermatozoa are required for the fertilization of a few ova not to assure the chance meeting of a sperm and ovum but rather to supply a sufficient concentration of hyaluronidase as a prerequisite for fertilization in the immediate environment of the sperm and ovum (7). This theory is further strengthened by the recent finding of more direct evidence indicating that the spermatozoa themselves produce the hyaluronidase content of semen and that the amount of hyaluronidase present is positively correlated with the number of spermatozoa (12, 13). A corollary to the theory, likewise, has been inferred by most investigators of the problem that seminal specimens containing greater amounts of hyaluronidase possess greater fertilizing capacities and that addition of the enzyme to semen in cases of oligospermia will increase the chances of fertilization.

In accord with the above observations, we have found hyaluronidase titers in cattle to be positively correlated with spermatozoa counts. Our results indicate, however, that above some hyaluronidase threshold value for fertilization, which at present remains obscure, increased amounts of hyaluronidase are associated with decreased fertilizing capacities. Thus hyaluronidase titer and percentage fertility have a net correlation coefficient of −.41999, a highly significant figure. A physiological explanation of this negative effect cannot be demonstrated with the available knowledge but an excess of the enzyme might produce an effect undesirable for fertilization on the ovum itself. In this respect it is interesting to note that Pincus and Enzmann (1) observed that rabbit semen which dispersed the cumulus cell mass of ova most speedily and had the greatest number of sperm per cmm. completely dissolved the ova after 24 hours.

It has been observed by several investigators that beyond a particular minimal range, increase in the number of spermatozoa used for insemination yields progressively higher percentages of fertilizations, until an optimal count is reached. Apparently beyond this optimal spermatozoa count, no further increase in the proportion of fertilizations accomplished is noticeable. The latter situation is the one encountered with normal bull semen as is demonstrated by the fact that the correlation of percentage fertilization with sperm count ($r_{AC} = +.24436$) which we obtained was not significant. However, since hyaluronidase titer is positively correlated with sperm count and, beyond a particular minimal value, progressive amounts of hyaluronidase are associated with decreased fertilization percentages, it may well be that numbers of spermatozoa, greater than the so-called optimal count, may still be capable of producing higher fertilization percentages but that their influence is nullified by the associated increase in hyaluronidase content. Indeed just such a condition would seem to be operative. When the influence of hyaluronidase content is kept constant, the correlation between percentage fertilization and sperm count becomes highly significant ($r_{AC.B} = +.36730$). Evidently the concept that large numbers of sperm serve to increase the mathematical chances of a sperm meeting the ovum must be at least in part retained unless another as yet unidentified factor, associated quantitatively with spermatozoa in fertilization, is assumed.

Clearly, any examination of the fertilizing capacity of semen must take into consideration the dual antithetical relationships of sperm count and hyaluronidase content. The quantitative effects of these two variables upon fertilization are given by the multiple regression equation coefficients. Any attempt to establish optimal and minimal sperm concentrations for fertilization, therefore, should be referable to a constant minimal value of hyaluronidase. Similarly, the establishment of a threshold amount of hyaluronidase for fertilization should be based on a constant number of spermatozoa. For insemination purposes, the ideal semen specimen should contain a maximum number of spermatozoa and the smallest hyaluronidase content still capable of preparing the ovum for fertilization.

Since dilution of a semen specimen does not alter the ratio of hyaluronidase to sperm numbers, there will be no significant change in the percentage fertilization obtained with dilution up to a point. However, when dilution becomes great enough so that the sperm count falls below the threshold number for the amount of hyaluronidase present or, conversely, when the hyaluronidase titer becomes inadequate for fertilization, percentage fertilization will be materially affected. In this regard Salisbury (14) found that, in the insemination of 5765 cattle, dilutions of 1:40, 1:60, 1:80 and 1:100 of the same semen specimen gave no significant differences in percentage fertilization. Equation V may be used for predicting percentage fertilization in cattle. However, for use with highly diluted semen it must first be ascertained whether sperm count and/or hyaluronidase titer fall below threshold values required for the process of fertilization.

In a few cases of human infertility characterized by oligospermia, it is possible that the addition of hyaluronidase might result in conception. In many more instances of infertility where the semen is incriminated, however, it is likely that too great an amount of hyaluronidase is already present in relation to the number of spermatozoa. In cases such as these, the development of methods for reducing the hyaluronidase content without affecting the spermatozoa present may well lead to an increased conception rate.

SUMMARY

1. Spermatozoa count was found to be significantly correlated with hyaluronidase titer and with percentage fertilization in cattle.

2. Hyaluronidase titer, beyond a threshold amount, has been found to have a relatively high negative correlation with percentage fertilization.

3. A multiple regression equation for percentage fertilization, with sperm count and hyaluronidase titer as the two independent variables, has been derived.

4. Criteria for the establishment of minimal and optimal sperm concentrations and of an ideal seminal specimen for fertilization have been set forth.

5. The use of a predictive equation for percentage fertilization in the insemination of cattle, along with the probable effect of semen dilution, is suggested.

6. The relation of hyaluronidase to the possible therapeutic treatment of certain types of human male infertility was indicated.

REFERENCES

1. Pincus, G. and E. V. Enzmann. J. Exper. Zool. **73:** 195, 1936.
2. McClean, D. and I. W. Rowlands. Nature **150:** 627, 1942.
3. Fekete, E. and F. Duran-Reynals. Proc. Soc. Exper. Biol. and Med. **52:** 119, 1942.
4. Werthessen, N. T., S. Berman, B. E. Greenberg and S. L. Gargill. Am. J. Urol. **54:** 565, 1945.
5. Leonard, S. L., P. L. Pearlman and R. Kurzrok. Endocrinology **39:** 261, 1946.
6. Walton, A. Roy. Soc. London Proc. **101B:** 303, 1927.
7. Rowlands, I. W. Nature **154:** 332, 1944.
8. Hotchkiss, R. S., E. K. Brunner and P. Grenley. Am. J. Med. Sci. **196:** 362, 1938.
9. Magath, T. B., J. Berkson and M. Hurn. Am. J. Clin. Path. **6:** 568, 1936.

0. McClean, D. Biochem. J. **37:** 169, 1943.

1. Rogers, H. J. Biochem. J. **39:** 435, 1945.

2. Hechter, O. and Z. Hadidian. Endocrinology **41:** 204, 1947.

3. Swyer, G. I. M. Biochem. J. **41:** 409, 1947.

4. Salisbury, G. W. J. Dairy Sci. **29:** 695, 1946.

EFFECTS OF CERTAIN FACTORS UPON THE LEVEL OF THE PLASMA PHOSPHATASES OF BREEDING BULLS[1]

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Though numerous studies have related the plasma alkaline phosphatase level of human beings and experimental animals to the metabolism of calcium and phosphorus, many recent investigations show that variations in the phosphatase level of plasma and other tissues may be accompanied or caused by a number of different physiological and pathological conditions.

The results of a limited number of investigations of the plasma phosphatases of the bovine have been variable. Allcroft and Folley (1) reported a wide range of serum phosphatase concentrations in cows with the activity somewhat increased during pregnancy, whereas Auchinachie and Emslie (2) found the opposite trend. Although no relationship was found between the milking capacity and the enzyme concentration, the enzyme level decreased with advancing age until maturity was reached (1). Wilson and Hart (3) demonstrated a high plasma phosphatase level three weeks or more before calving with a decrease near the time of parturition followed by a tendency to increase. A similar general observation was reported by Hibbs *et al.* (4). Phillips (5) demonstrated that the plasma phosphatase of cattle suffering from fluorine toxicosis increased in proportion with the fluorine intake and indicated that the enzyme offers a sensitive test for this disturbance. The plasma calcium and phosphorus remained within normal limits. No data are reported on the plasma phosphatase of bulls.

The object of this investigation was to study the relationships of diet, semen production, plasma calcium and phosphorus and age to the plasma phosphatases of dairy bulls.

EXPERIMENTAL

Twenty-three bulls from which semen was obtained at varying rates were employed in this study. Eleven of these animals constituted two groups in a nutrition experiment in which six bulls received a complex concentrate mixture containing supplemental minerals and vitamins and five bulls were fed a simple concentrate mixture consisting largely of corn with no mineral or vitamin supplementation. These animals received the same average grade mixed hay at the rate of 1 lb. per 100 lb. body weight. The concentrate mixtures were fed in sufficient quantity with hay to supply 1.18 lb. of digestible nutrients per 100 lb. of body weight daily. No more than three weeks difference existed in the age of the 11 bulls used in this phase of the study. These bulls were 27 months old at the beginning and 33 months old at the termination of the experiment.

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The remaining 12 bulls ranging from 17 to 128 months of age were employed in an artificial breeding stud and were fed in a manner considered to be consistent with the production of high quality semen.

The activity of the alkaline and acid phosphatases was determined in plasma at pH 9.3 and 5.0, respectively, by the method of King and Armstrong (6) as modified by Wiese *et al.* (7). A unit of phosphatase was defined as one mgm. phenol liberated from the disodium phenyl phosphate substrate in 30 minutes at 37°C. by 100 ml. plasma.

To facilitate quantitative expression, the average daily number of ejaculates, volume of semen and number of spermatozoa were calculated from the total number of ejaculates and spermatozoa and volume of semen obtained during the arbitrarily selected period of three months, terminating with the estimation of the plasma phosphatases.

TABLE 1. EFFECT OF REST UPON THE PLASMA ALKALINE PHOSPHATASE LEVEL OF BREEDING BULLS

BULL NO	DURING HEAVY USE	AFTER 52-DAY REST	INCREASE
	units	*units*	*units*
2	6.88	11.60	4.72
3	3.37	10.75	7.38
4	2.27	9.31	7.04
11	5.27	10.84	5.57
13	5.03	10.59	5.56
6	7.13	13.07	5.94
9	4.43	10.58	6.15
10	3.50	10.03	6.53
12	4.80	10.51	5.71
14	6.60	12.14	5.54
Average	4.93	10.95	6.02

RESULTS

Effect of diet on plasma phosphatase level. Since numerous reports indicate a possible relationship between the nutrition of animals and the alkaline phosphatase activity of their plasma, the concentration was measured in the plasma of two groups of bulls receiving extremely different concentrate mixtures.

Since these rations did not appear to affect the acid or alkaline phosphatase concentration of the plasma of these bulls, individual group data for phosphatase levels and data relative to the composition of diet are not given.

Effect of rest on plasma phosphatases of breeding bulls. It was observed that the plasma of bulls used for semen production sparingly or not at all contained higher concentrations of alkaline phosphatase than bulls ejaculated more frequently.

Table 1 shows a 2.2-fold increase in the alkaline phosphatase upon resting (for 52 days) bulls which had been yielding an ejaculate at 3 to 5-day intervals. The act of resting bulls from semen collection had no perceptible effect on the activity of the plasma acid phosphatase.

Relationship of rate and quantity of semen ejaculation to plasma phosphatases. An examination was made of the relationship of the frequency with which semen was obtained, the volume of semen and concentration of spermatozoa produced to the plasma phosphatases. The quantitative expression of the average daily figures used in these relationships appeared to be justified since semen was obtained at fairly regular intervals regardless of the rate.

Table 2 demonstrates the effect of taking semen on the plasma alkaline phosphatase of a bull from which semen had not previously been obtained. The collection of nine samples of semen totaling 193 ml., obtained by massaging the ampullae of the ductus deferens and seminal vesicles during a 16-day period, effected an abrupt decline in enzyme concentration from 18.95 (range 18.04 to 19.75 in five samplings) to 5.24 units per 100 ml. plasma.

TABLE 2. DECLINE OF PLASMA ALKALINE PHOSPHATASE LEVEL IN A BULL FROM WHICH SEMEN HAD NEVER BEFORE BEEN OBTAINED

PHOSPHATASE BEFORE SEMEN COLLECTION	SEMEN COLLECTION PERIOD	SAMPLES OBTAINED	SEMEN VOLUME	SPERMATOZOA NO.	PHOSPHATASE AFTER SEMEN COLLECTION
units	*days*	*no.*	*ml.*	*billions*	*units*
18.95 (18.04–19.75)[1]	16	9	193	4.14	5.24

[1] Range found in five samplings during a 6-month period.

TABLE 3. EFFECT OF FREQUENCY AND QUANTITY OF SEMEN EJACULATION UPON PLASMA ALKALINE PHOSPHATASE LEVEL

	PHOSPHATASE RANGE (UNITS)				CORRELATION COEFFICIENT AND STANDARD ERROR
	2.00–5.99	6.00–9.99	10.00–13.99	14.00–19.75	
Ejaculates/day	.26	.28	.10	.01	−0.71 ±0.06
Volume/day (ml.)	1.28	1.04	.43	.05	−0.83 ±0.04
Spermatozoa/day × 10^8	13.26	11.11	4.32	.57	−0.86 ±0.03
No. cases	13	21	25	6	

A marked decline in plasma alkaline phosphatase concentration was found to accompany an increase in the ejaculation rate and in the volume of semen and number of spermatozoa ejaculated. The relationships are established in table 3 and figure 1. The degree of relationship of the plasma alkaline phosphatase level was highest with the number of spermatozoa ejaculated, intermediate with the volume of semen ejaculated and lowest with the number of ejaculates yielded. All of these were highly significant statistically.

The concentration of plasma acid phosphatase did not appear to be related to the rate or quantity of semen ejaculated.

Relationship of plasma calcium and phosphorus and age to plasma phosphatases. The extensive use of plasma alkaline phosphatase values in the detection of disturbed bone metabolism and the decline accompanying aging, found by a number of workers, made it necessary to establish whether or not the observed differences in enzyme concentration of bulls' plasma were associated with the levels of plasma calcium and phosphorus or the age of bulls. It was found that the calcium and phosphorus concentrations in the plasma of these animals were not greatly different regardless of the alkaline phosphatase activity and that age was without appreciable effect upon the enzyme level.

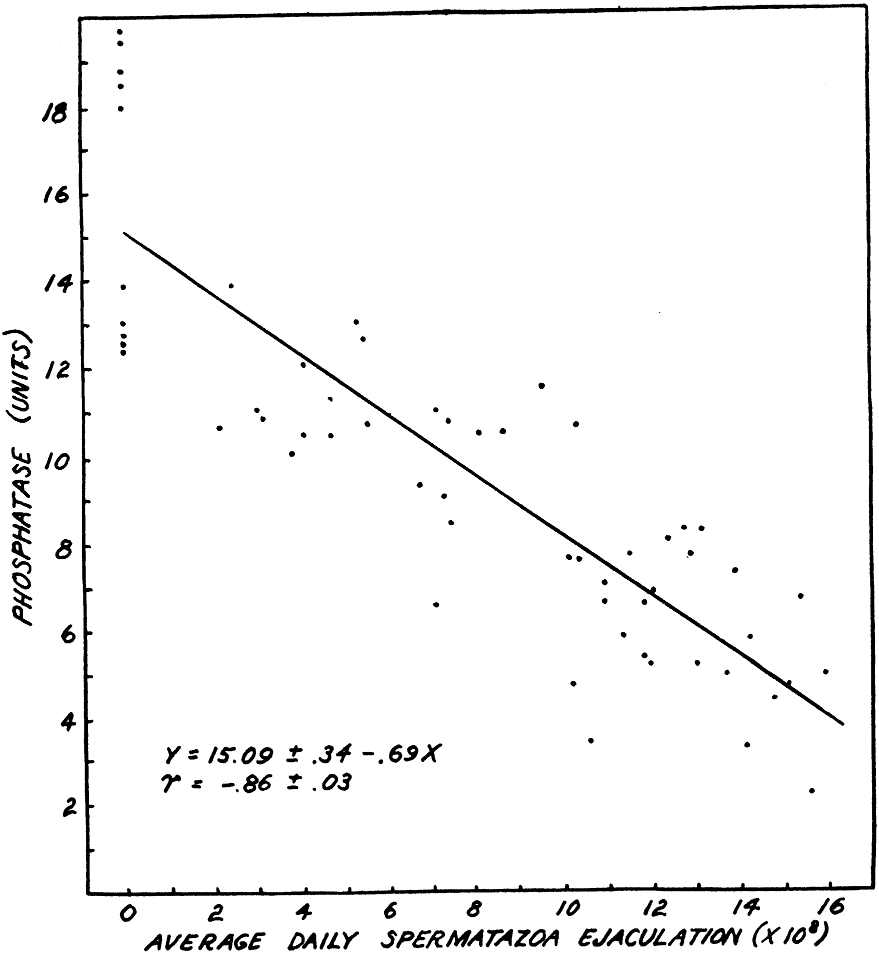

Fig. 1

The acid phosphatase concentration did not bear any noticeable relationship to the plasma levels of calcium and inorganic phosphorus or age.

DISCUSSION

Although dietary influences on the plasma alkaline phosphatase are widely known, the limits of the rations fed in this experiment were without effect upon the activity of the enzyme in the plasma of bulls. The complex concentrate feed used in this study provided 3.62 grams more Ca, 1.91 grams more P, 40 mgm. more Fe, 73 mgm. more Mn, 6 mgm. more Cu, 0.45 mgm. more Co, 37.10 grams more protein, 11.16 grams more fat, 13,620 I. U. more vitamin A and 2,043 U. S. P. units more vitamin D per pound than was supplied in the same amount of the simple concentrate feed. The literature demonstrates the effects of certain of these dietary factors upon the phosphatase level of plasma and other tissues of humans and experimental animals; however, in view of the great differences of the two rations employed in this investigation, the possibility of demonstrating a dietary influence on the concentration of plasma phosphatases of breeding bulls seems remote.

Since it is known that cows in widely different mineral balances seem to maintain similar plasma levels of calcium and phosphorus, these data involving plasma calcium and phosphorus should not be interpreted to mean that the mineral metabolism of the bull is not related to alkaline phosphatase.

The observation of a declining phosphatase concentration with aging in experimental animals (1) and humans (8) may be a secondary reflection of bone metabolism. The small number of very young and old bulls available for study limited the age range used in this investigation; however, it was found that the plasma of new-born bull calves contained much greater quantities of the alkaline phosphatase than did that of older bulls (9).

Although the plasma alkaline phosphatase decreases in a consistent manner with increased rates of semen ejaculation and quantities of semen yielded, these data do not allow the mechanism involved in the decline of the enzyme in plasma to be ascertained. It seems indicated, though, that the semen alkaline phosphatase may be derived from the blood at which expense the enzyme level may be maintained in the semen. The work of Gutman and Gutman (10) shows that the concentration of alkaline phosphatase in the seminal fluids of man is very low and, in this respect, is opposite to acid phosphatase. The results of a few determinations conducted in this laboratory would indicate a very high concentration of alkaline phosphatase in bull semen (9). A study of the enzyme in bull semen will be published later.

It is not known what practical husbandry application these findings may have; however, if a sufficiently large number of bulls were examined, it appears probable that these data could be used as an index of when a rest from the breeding routine might be beneficial to bulls.

SUMMARY

1. The plasma alkaline phosphatase concentration of bulls appears to be largely dependent upon the frequency of semen ejaculation and, more particularly, upon the number of spermatozoa yielded.

2. The rations used in this investigation did not elicit any perceptible influence on the plasma phosphatases of bulls.

3. No relationship was observed between the plasma level of alkaline or acid phosphatase and the plasma levels of calcium, inorganic phosphorus or age of bulls.

4. The level of acid phosphatase was not affected by the rate of semen collection or quantity of semen ejaculated.

5. In 144 analyses made on the plasma of bulls, the average alkaline phosphatase activity was 8.98 (range 1.99–21.03) units per 100 ml. and the average acid phosphatase was 2.09 (range 0.35–6.63) units per 100 ml.

REFERENCES

1. ALLCROFT, W. M. AND S. J. FOLLEY. Biochem. J. **35:** 254, 1941.
2. AUCHINACHIE, D. M. AND A. R. G. EMSLIE. Biochem. J. **27:** 351, 1933.
3. WILSON, L. T. AND E. B. HART. J. Dairy Sci. **15:** 116, 1932.
4. HIBBS, J. W., W. E. KRAUSS, W. D. POUNDEN, C. F. MONROE AND T. S. SUTTON. J. Dairy Sci. **29:** 767, 1946.
5. PHILLIPS, P. H. Science **76:** 239, 1932.
6. KING, E. J. AND A. R. ARMSTRONG. Can. Med. Assoc. J. **31:** 376, 1934.
7. WIESE, A. C., B. C. JOHSON, C. A. ELVEHJEM, E. B. HART AND J. G. HALPIN. J. Biol. Chem. **127:** 411, 1939.
8. STEARNS, G. AND E. WARWEG. J. Biol. Chem. **102:** 749, 1933.
9. REID, J. T., G. M. WARD AND R. L. SALSBURY. Unpublished data. 1947.
10. GUTMAN, A. B. AND E. B. GUTMAN. Endocrinology **28:** 115, 1941.

UTILIZATION BY DOGS OF THE NITROGEN OF HEATED CASEIN

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Earlier investigations have shown that heat treatment of proteins with temperatures over 120° appreciably lowers their nutritive value as determined by rat growth and balance methods (1–4). Morgan, Greaves and Loveen (5) reported that the growth value of casein for rats was decreased 17 per cent when heated at 140° C. for 30 minutes, but that its digestibility was lowered only 4 per cent. They concluded that lysine was the first amino acid damaged and histidine the second when casein was heated to this temperature. Arnold and Elvehjem (6) observed running fits in dogs fed a heated diet of wheat and meat scraps and noted some improvement when lysine was given. No other work with dogs fed heated proteins has been reported. This study was undertaken largely to determine whether the response of rats to heated diets can be duplicated in another species.

Two litters of mongrel dogs and two litters of purebred cocker spaniels, a total of 17 dogs, were used in this experiment. Some of each group were placed on a diet containing unheated casein at the same level used for the littermates fed the diet containing heated casein. The effect of these regimes was noted on the growth, blood composition, nitrogen utilization and tissue changes.

METHODS

The same type of casein was used in all the experiments, an acid-precipitated, dried commercial product designated raw casein, and the same heated for 15 or 30 minutes in layers one to one and one-half inches deep at 130°, 140° or 200°. The temperature of the casein was carefully controlled by preheating the oven to a higher temperature than that desired so that the introduction of the amount of casein prepared at one time brought the material to the desired temperature within 10 minutes. The oven temperature was then controlled so that the casein remained at the desired temperature for either 15 or 30 minutes. In this way all lots of casein prepared for a given experiment were subjected to identical treatment. The casein heated at 130° and 140° was light yellowish buff in color and that heated at 200° a considerably darker shade. The depth of color was found to vary quite consistently with the temperature to which the casein was exposed.

The animals assigned to the heated diet were started on a casein heated at 130°. Since this did not seem to slow their growth measurably, the temperature of heating was increased to 200° after 46 days. This diet produced such severely deleterious effects that in the later experiments a lower temperature, 140°, was used. The diets were made up of casein, raw or heated, 18, 24 or 36, $CaCO_3$ 1.5, hydrogenated cottonseed oil 10.0, salt mixture #5,[1] 2.4, cornstarch and sucrose in equal amounts to make the total, 100. In addition each dog received one gram yeast and 1 gram wheat germ per kgm. per day except preceding and during periods of collection of excreta, when they were fed the crystalline vitamins, per kgm. per day, thiamine, pyridoxine, and riboflavin each 0.1 mgm., pantothenic and nicotinic acid each 2 mgm. Grayfish oil fortified by irradiated ergosterol was fed to supply vitamins A and D in the amount of 400 I.U. of vitamin A and 50 I.U. of vitamin D per kgm. per day. The protein supplied by the yeast and wheat germ amounted to 7 to 18 per cent of the total protein fed, the proportion increasing with the lowering of intake in the older dogs.

The dogs were kept in individual standard metabolism cages. From the time they were weaned at five to seven weeks of age until they were four months old, they were given the weighed daily ration in two feedings. After they were four months old, the ration was given in one feeding daily. The dogs on the unheated diet ate their food promptly and completely, as did usually also those on the heated diet. The animals in each group were paired, one of the pair being placed on the raw and the other on the heated diet, and each two animals thus paired were fed daily the same amount of nitrogen and total food, regardless of differences in weight increases. The amount of food given decreased from 200 cal/kgm /day at first to 80 cal/kgm/day when the dogs' growth rate levelled off after seven to eight months.

Total serum protein and serum albumin were determined by the micro-Kjeldahl method, and nonprotein nitrogen was similarly measured on the Folin-Wu filtrate. Serum albumin and globulin were fractionated by the method of Kingsley (7) except in some of the earliest determinations in which Howe's method (8) was used. Simultaneous determinations by the two methods indicated that comparable results could be obtained. Because of its rapidity, Kingsley's method was chosen.

The nitrogen content of pooled five-day urine and fecal collections was measured by macro-Kjeldahl determinations. The urine was preserved with a small amount of acid and toluene and the feces were dried daily. Diarrhea, particularly on the heated diets, sometimes interfered with the fecal collections, and in these instances less than five-day collections were used.

Creatine was determined in the muscle tissue of the dogs of group II by the method of Rose, Helmer and Chanutin (9). Vitamin A was determined in the livers of this group by the method of Davies (10) and in the serum by the method of Yudkin (11).

EXPERIMENTAL RESULTS

Growth. As shown by the growth curves (fig. 1, 2 and 3), the dogs fed the heated diet made poorer growth than the littermates fed the same amounts of the raw diet with only one exception, *dog 13* of group III, which was heavier than his control *dog 14*, at the start. The stunting was corrected by rapid compensatory growth whenever an animal was changed from the heated to the raw diet, as was done in the case of *dog 8*, and the reverse condition occurred when the heated was substituted for the raw diet as in the cases of *dogs 9* and *3*.

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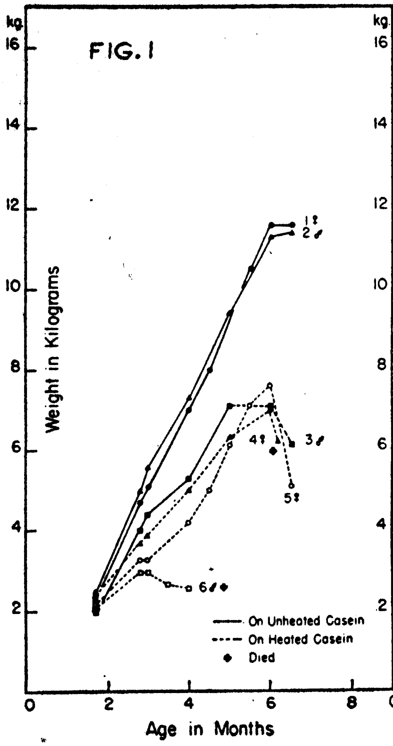
[1] Axelrod, H. E., A. F. Morgan and S. Lepkovsky. J. Biol. Chem., **160**: 155, 1945.

There was less severe depression of growth in the group (III) fed the casein heated at 140° than in the two groups fed the more highly heated diet. Three of the former group were given the diet containing 24 per cent casein and four received an 18 per cent casein diet. Two of those fed the 24 per cent diet, *dogs 16* and *17*, were of a different litter from the other five, and grew less rapidly, but the difference between the dogs on the raw and heated diets remained the same. When *dog 9* of group II was changed from a diet containing 18 per cent heated casein to one containing 36 per cent heated casein, her condition rapidly deteriorated.



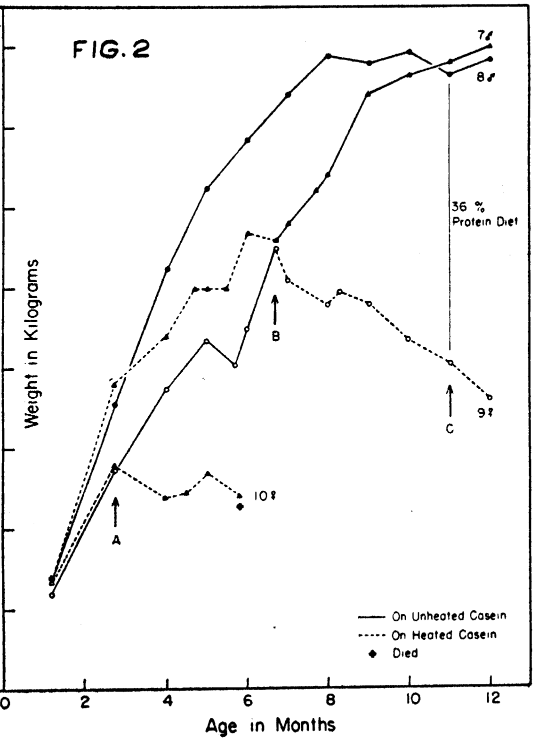


FIG. 1. GROWTH OF GROUP I, hounds, fed from weaning purified diets containing 18 per cent casein, unheated or heated 15 minutes at 200°.

FIG. 2. GROWTH OF GROUP II, shepherd dogs, fed from weaning on purified diets containing 18 per cent casein, unheated or heated. At first the casein was heated 30 minutes at 130°, but at point A was changed to casein heated 15 minutes at 200°. At B, *dog 7* was changed from heated to unheated, and *dog 9* from unheated to heated diet. At C, the level of casein in both diets was raised to 36 per cent.

Three measures were tried in an attempt to improve the growth of the dogs on the heated diets: 1) the addition of 1-lysine dihydrochloride[2] to the experimental diet, 2) the similar addition of lactalbumin, a commercial product and 3) the substitution for the experimental diets of the stock diet. *Dogs 5*, *6* and *17* were

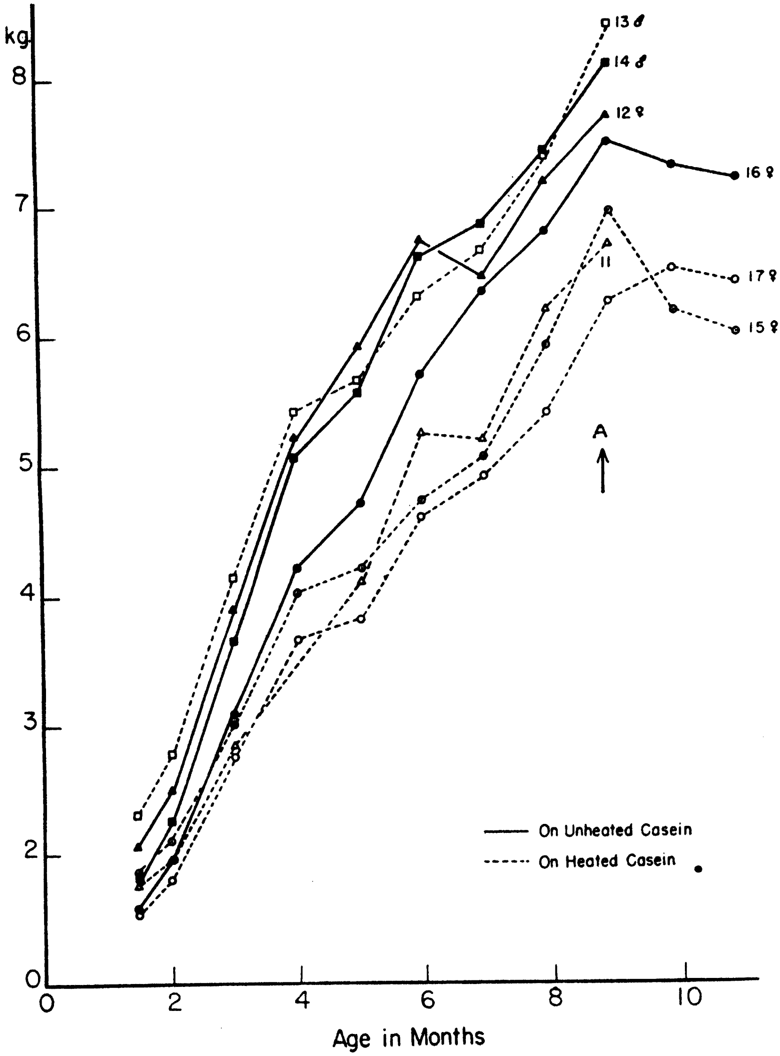


FIG. 3. GROWTH OF GROUP III, cocker spaniels, fed from weaning on purified diets containing 18 or 24 per cent casein, unheated or heated 15 minutes at 140°. *Dogs 15*, *16* and *17* received the 24 per cent diet, the others, 18 per cent. At A, force feeding of *15* and *17* was discontinued.

given the lysine; *dog 6* after 80 days by subcutaneous injection, 0.5 gram daily for 10 days; *dog 5* after 135 days, 1 gram daily by mouth for 10 days; and *dog 17* after 135 days, 2 grams daily for eight days by mouth, and, 23 days later when more lysine became available, 1 gram daily for 20 days. The amounts given in the cases of *dogs 5* and *6* were apparently too small to be of advantage, but *dog 17* made some improvement in growth, in serum protein levels and in nitrogen retention during, but more markedly in the period after, the lysine administration. The nitrogen loss by *dog 5* was also decreased somewhat in the period following the lysine feeding.

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[2] The lysine was generously supplied by Merck & Co., Rahway, New Jersey.

Dogs 13 and *14* of group III after five months were each given in addition to their usual diet 3 grams dry lactalbumin daily, amounting to 60 mgm. nitrogen per kilo per day added to the 213 to 220 mgm. per kilo per day contained in their basal diet. During the two months that this supplement was given the growth, nitrogen retention and serum protein level of *dog 13* on heated diet were improved, but there appeared to be no change in *dog 14* on the unheated diet.

All seven of the dogs of group III were placed on the 36 per cent casein stock diet after 7 to 13 months on the experimental diets. All the dogs which had received the heated diets at once improved in weight, condition of coat, activity and appetite.

The animals in group III which received the less severely heat-treated casein had few signs of inferiority other than slow growth, although their hair was dry and short as compared with their controls receiving the unheated diet. In groups I and II, however, the dogs on the diet containing casein heated at 200° had scaly dry skin, dull short hair and recurring skin lesions, accompanied by loss of hair, particularly about the eyes. Two dogs in group I and one in group II died during the experiment, apparently because of the deficiency.

Serum proteins. In all cases the levels of total serum proteins and serum albumin of the dogs fed the heated casein were lower than those of the corresponding animals on the unheated protein. There were larger differences shown by the groups fed the more severely heated diets, as illustrated in figure 4. The level of circulating serum proteins and albumin found after three to five months on the diets in groups I and II showed depression of both values in *dogs 5*, *4* and *9* as compared with *dogs 1*, *2* and *8*, their pair-fed controls on the unheated protein. The changes in these levels produced in two months in *dog 3* when removed from the unheated to the heated diet and in three months in *dog 7* when changed from the heated to the unheated diet were striking and consistent. In figure 5 is shown the decrease in the serum protein level of *dog 9* which followed the change in the level of heated protein in the diet from 18 per cent to 36 per cent, and the fall in all these serum values when the three dogs of group II were placed on low-nitrogen diets approximating the endogenous level.

The dogs of group III which received the less severely heated diet also had total serum proteins and albumin levels slightly lower than had their controls on the unheated diets. The group fed the 18 per cent casein diets had about the same serum protein levels as those fed the 24 per cent casein diets (fig. 6), and the differences between those on the heated and unheated protein appeared to be of the same order. However, *dogs 15* and *17* fed a heated 24 per cent diet maintained somewhat higher serum albumin than *dogs 11* and *13* fed the heated 18 per cent diet. When *dogs 13* and *14* were each given three grams lactalbumin per day, a rise in serum protein level occurred in *dog 13*, but little change in albumin level. When all four dogs of this group were placed on the 36 per cent unheated casein stock diet, in two to three months all had attained the same serum protein and albumin levels. When l-lysine dihydrochloride was given *dog 17*, 40 grams in 52 days, the serum protein and albumin levels rose perceptibly above those of the pair-fed *dog 15* on unsupplemented similar diet but did not equal the levels shown by the animal, *dog 16*, given the unheated protein. These three dogs were maintained on the experimental diets several months longer than any of the others, and it was noted that after maturity was reached, at 10 to 12 months of age, the differences in serum protein levels tended to decrease.

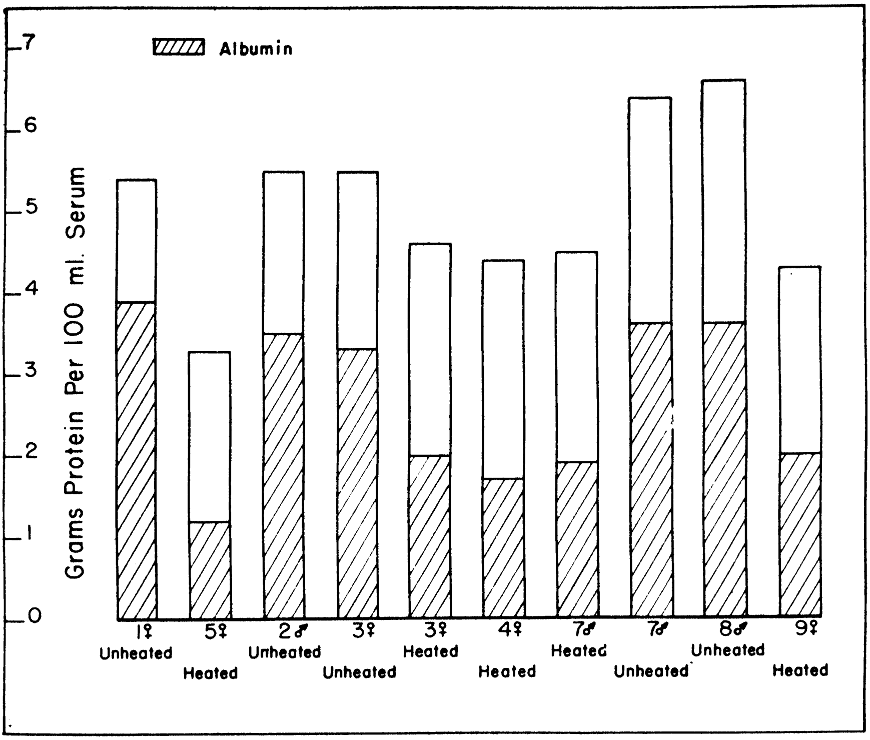
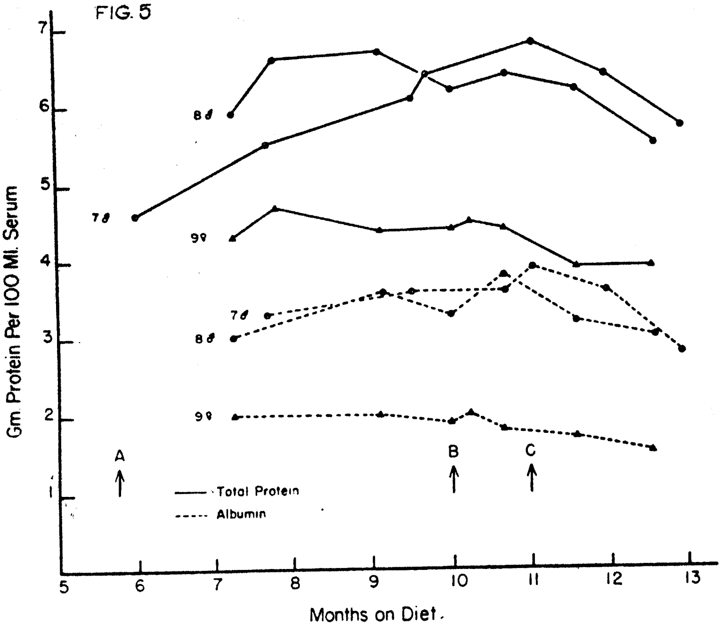


FIG. 4. SERUM PROTEIN AND ALBUMIN as affected by heating of dietary casein. The number of the dog is given below the columns. These columns represent the blood protein values after two to five months on the diets. *Dogs 2* and *8* were on unheated diet throughout, *dogs 5* and *4* on heated (200° for 15 minutes) casein throughout. *Dog 3* was first on unheated diet for three to four months, then heated diet for two months. *Dog 7* was on heated diet for six months then unheated diet for six months. *Dog 9* had been on heated diet for three months at this time.

Hemoglobin. From the fourth to the eighth month on the diet the dogs of group III on heated diet had 11.6 to 14.9 grams hemoglobin per 100 ml. blood and those on the unheated diet had 13.2 to 15.8. The per cent of packed cells



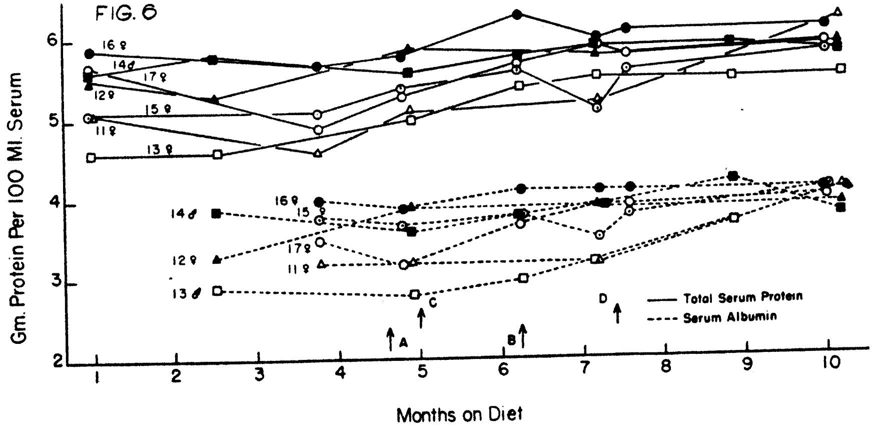


FIG. 5. SERUM PROTEIN AND ALBUMIN LEVELS OF GROUP II. At A, *dog 7* was changed from 18 per cent heated casein (200° for 15 minutes) to the unheated diet, and *dog 9* from unheated to heated diet. At B, *dogs 7* and *8* were changed to unheated casein, 36 per cent, and *dog 9* to heated casein, 36 per cent. At C all three were placed on the nonprotein diet, followed by intakes at the endogenous level.

FIG. 6. SERUM PROTEIN AND ALBUMIN LEVELS OF GROUP III. At C began 3 grams lactalbumin daily administration to *dogs 13* and *14*, continued to D. Between A and B, 40 grams dl-lysine hydrochloride was given *dog 17*. At D, *dogs 11, 12, 13* and *14* were changed to 36 per cent unheated casein diet.

was also usually larger in the latter. The dogs of groups I and II which were fed the severely heated casein after three to five months on the diet had only 8.4 to 9.6 grams hemoglobin per 100 ml. blood while their pair-fed controls on the unheated diet had 11.7 to 14.7.

Nonprotein nitrogen. The level of blood nonprotein nitrogen varied very little throughout the feeding period. The dogs in all groups which were fed the unheated protein had 30 to 38 mgm. per cent and those fed the heated diet 17 to 28 mgm. per cent.

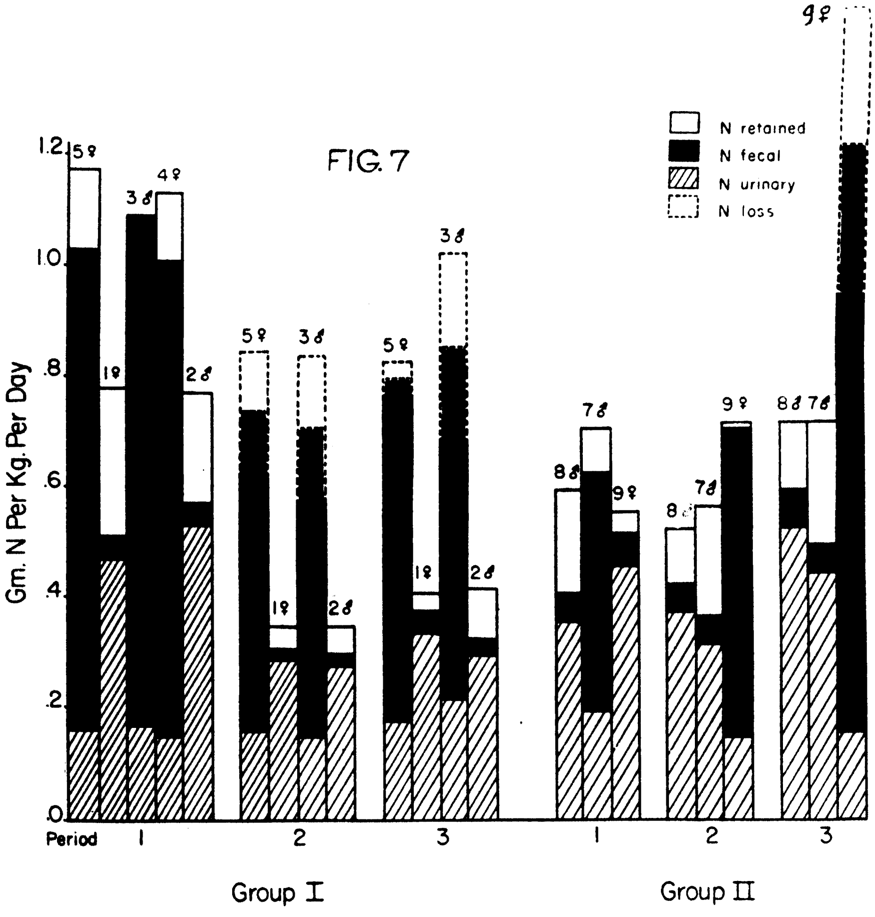


FIG. 7. NITROGEN BALANCES OF DOGS OF GROUPS I AND II. *Group I*, periods 1, 2 and 3, one balance each after 125, 139 and 146 days on the diet. *Group II*, period 1, two balances in the fourth month on the diets; period 2, three balances in the seventh and eighth months; period 3, one balance in the ninth month. Between periods 1 and 2, *dog 7* was shifted from heated to unheated diet and *dog 9* from unheated to heated diet.

Nitrogen balances. The intake of the dogs on the heated diet was kept parallel with that of their pairmates on the unheated protein during the balance periods by force feeding when necessary. These intakes were kept nearly equal regardless of differences in the weight of the animals. Each collection period was five days in length. Occasionally the dogs on heated diet excreted very soft fecce, thus interrupting the collections.

As shown in figure 7 a large part of the nitrogen excreted appeared in the feces of the dogs of group I fed the casein heated at 200°. In succeeding periods the nitrogen retention of these animals decreased and eventually became negative. The proportion of food-nitrogen lost in the feces increased from 44, 56 and 47 per cent in the first period, to 74, 84 and 76 in the second and 94 and 96 in the third. The two dogs on the unheated casein excreted in the feces only 5 to 10 per cent of the food nitrogen throughout the experiment. During the balance periods the latter dogs weighed 10 to 11 kgm., the former 5 to 7 kgm.

An attempt at determination of the endogenous level of nitrogen excretion was made with the three dogs of group II after they had been on the diet ten months. When fed nonprotein basal diet, after a five-day adjustment period, the urinary nitrogen excretion of the two dogs which had been fed the unheated casein was found to be similar, and their fecal output very small (table 1). The dog which had been subsisting on the heated diet excreted equal amounts of nitrogen in urine and feces. The pattern established on the heated protein regime apparently persisted. The dogs were then returned to their original casein diets but at approximately the endogenous level. The true digestibility of the unheated casein appeared to be 89 and 87 per cent and of the heated casein 30 per cent.

TABLE 1. THE TRUE DIGESTIBILITY OF HEATED (200° FOR 15 MIN.) AND UNHEATED CASEIN

In mgm. per kgm. per day

DOG NO.	WEIGHT	PROTEIN FED	N INTAKE	URINE N	FECAL N	BALANCE N	ABSORBED N	TRUE DIGESTIBILITY
	kgm.							*per cent*
8♂	15.4	No protein	—	110	6	−116		
	13.0	Unheated casein	154	164	22	−32	138	89
7♂	15.7	No protein	—	113	11	−124		
	14.0	Unheated casein	143	145	30	−32	124	87
9♀	7.1	No protein	—	60	59	−119		
	5.9	Heated casein	170	52	178	−60	51	30

Earlier balance experiments with these three animals indicated large losses of nitrogen in the feces when the heated protein was fed, with greatly increased excretion when the level of casein was raised from 18 to 36 per cent (fig. 7, period 3). *Dog 7*, which had been depleted by the earlier heated diet regime, retained more nitrogen in every period on the unheated casein than did *dog 8*, which had been fed the unheated diet from the beginning.

All of the animals of group III fed the casein heated at 140° for 15 minutes (fig. 8) exhibited the same digestive pattern as did those in groups I and II, but the loss of nitrogen in the feces was less, usually 30 to 40 per cent of the total nitrogen excretion, even after seven months on the diet. None of the four dogs of this group which were fed the heated casein showed the severe failure of nutrition seen in the animals fed the more highly heated casein. *Dog 17*, which was given l-lysine dihydrochloride in balance period 2 as previously mentioned, presented better nitrogen retention than *dog 15* on heated casein without added lysine in this period only.

Dogs 11 and *13*, fed the 18 per cent heated casein diets, were force-fed in period 1 to conform with the intakes of their controls, *dogs 12* and *14*, but the intake of the latter was decreased to the voluntary intake of *dogs 11* and *13* in period 2. It will be seen that the nitrogen retention was much decreased. In period 3, three grams lactalbumin was given daily to *dogs 13* and *14*, with accompanying improvement in the nitrogen retention of *dog 13*.

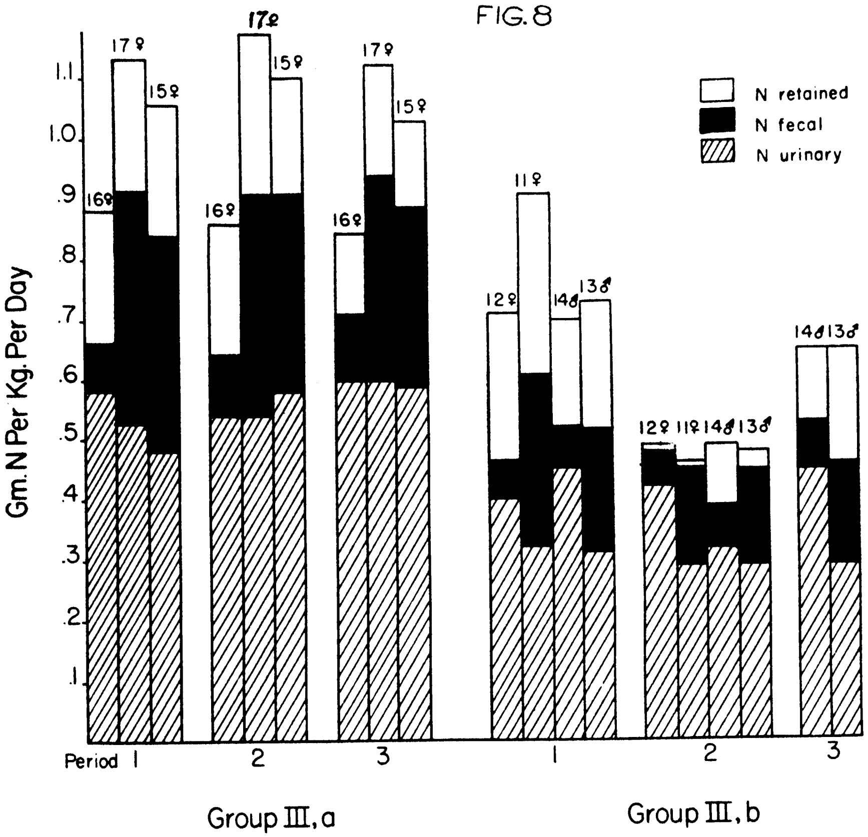


FIG. 8. NITROGEN BALANCES OF DOGS OF GROUP III. A. Dogs on 24 per cent casein diet. Period 1, six balances in the fourth and fifth months; period 2, three balances in the sixth month when 17 received one or more gram lysine daily; period 3, three balances in the seventh month. B. Dogs on 18 per cent casein diet. Period 1, two balances in the fourth month; period 2, one balance in the fifth month, no force feeding; period 3, three balances in the sixth month, when both dogs received 3 gram lactalbumin daily.

The retentions of these animals on heated diet during balance periods were often better than those of their controls on the unheated diet because of their greater intake in relation to body weight. The force-fed dogs sometimes made small gains in weight during the balance periods, but when the force feeding was discontinued ceased to gain or lost weight. Their voluntary intake was seldom lower per kilogram weight, however, than that of the normal animals. This persistence of positive nitrogen balance with stationary or decreasing body weight has been noted by Frost and Risser (12) and by Robscheit-Robbins, Miller and Whipple (13).

The apparent digestibility of the more severely heated casein was 28 per cent, that of the less severely heated casein 67 per cent, and that of the unheated casein 91 per cent.

Running fits. No running fits occurred in the dogs fed the unheated or the uncomplicated heated diets. Two weeks after *dog 17* was given lysine as previously indicated, the animal had three running fits on three successive days. Thereafter fits of varying severity occurred at irregular intervals during the remaining six months of this animal's life on the heated diet. Three days after *dog 13* was placed on the stock diet containing 36 per cent unheated casein, the animal had a running fit. That an improvement in diet could precipitate this symptom seems unlikely, yet only these two animals out of 11 fed heated casein were seen to undergo this condition. *Dogs 7, 11* and *15* were also changed from heated to raw or stock diet without occurrence of fits. Interest is attached to this symptom because there is some evidence that severely heated dog foods of low or poor quality protein content may induce such fits (14).

TABLE 2. ANALYSIS OF FRESH TISSUES OF DOGS FED HEATED (200° FOR 15 MIN.) AND UNHEATED CASEIN

DOG NO.	DIET	CONSTITUENT	HEART	MUSCLE	LIVER	BLOOD PLASMA (AT AUTOPSY)
8♂	Unheated diet, 383 days.	Water, per cent	78.5	77.6	—	—
		Nitrogen, per cent	2.80	3.16	—	
		Creatine, mgm. per cent	307	333	—	—
		Vitamin A, I.U. per gram	—	—	8640	1.08
7♂	Heated diet, 167 days, followed by unheated diet, 216 days.	Water, per cent	77.9	74.2	—	
		Nitrogen, per cent	2.82	3.32	—	
		Creatine, mgm. per cent	342	367	—	
		Vitamin A, I.U. per gram	—	—	6960	1.18
9♀	Unheated diet, 158 days, followed by heated diet, 215 days.	Water, per cent	78.7	76.4	—	
		Nitrogen, per cent	2.69	3.32	—	
		Creatine, mgm. per cent	268	293	—	
		Vitamin A, I.U. per gram			8240	0.35

Condition of tissues. Only the dogs in groups I and II were sacrificed. Exceedingly yellow livers in all the animals on heated diet were observed upon gross examination. The control livers were normal. Histological sections of the yellow livers made by staining with hematoxylin eosin showed vacuolization considered to be due to fatty infiltration, but stains for fat were not made. It is of interest to note that *dog 7*, which was changed from heated to raw diet, had a normal liver, which would indicate that any damage which occurred in this organ was reparable.

The gastronemious and heart muscles of *dog 9* had low creatine values in both cases but normal total nitrogen contents, as compared with the creatine and nitrogen of the tissues of the two dogs of the same group which had been fed unheated diet. The decrease in creatine may be due to methionine deficiency in the heated casein.

The vitamin A of the liver of *dog 9* was of the same order as that of the two normal animals but that of the circulating blood was low (table 2). These determinations[3] were made because *dog 9* had exhibited loss of hair, reddening and purulence of the eyelids, and skin eruptions which resembled the symptoms seen in vitamin A-deficient dogs. The utilization of vitamin A may have been impaired due to decrease in liver function, as suggested by Popper and Steigman (15), Ralli *et al.* (16) and others. This liver damage again may be referable to loss of lipotropic action in the heated casein due to changes in the methionine. Choline as such was not included in the diet but this was presumably supplied by the yeast and wheat germ used as the source of the B vitamins except during the actual nitrogen balance periods. The presence of normal vitamin A stores in the liver of *dog 9* with reduced plasma vitamin A level parallels conditions reported in clinical studies (17), and may be taken to mean that the damaged liver retained the vitamin in an inaccessible form or site (18).

DISCUSSION

The effect of heated casein on the metabolism of the dogs was shown by the decrease in rate of growth, the decrease in total serum protein and serum albumin, the decrease in nitrogen absorption, and the liver damage. Although true digestibility could not be determined in all cases, the large differences in apparent digestibility justify the conclusion that heating the protein seriously decreased its absorbability. It may also be concluded that the higher the temperature of heating of the casein, the greater the decrease in digestibility, since casein heated at 200° and fed at the 18 per cent level underwent a decrease in digestibility from 91 to 28 per cent, whereas that heated at 140° fell from 90 to 67 per cent. Little difference was apparent between digestibilities of the casein heated at 140° and fed at the 18 and 24 per cent levels, 68 and 66 per cents, respectively. To this lowered digestibility can probably be attributed most of the deleterious effects of the heated diet which are comparable with those of low protein diets.

It is well known that low protein diets may cause hepatic damage. Weech and Goettsch (19) who used a low protein diet of carrots and rice noted in dogs definite hepatic changes which they considered to be due to fatty infiltration. Elman and Heifetz (20) feeding the same type of carrot diet to dogs found extensive vacuolization of liver cells which was not due to accumulation of fat as shown by chemical analyses and stained sections, but which they ascribed to loss of liver protein. This is parallel with observations made on some of the livers of dogs in this study which showed vacuolization which may have been caused by fatty infiltration, but since no fat stains or chemical analyses were made, the assumption may be unwarranted. Since the liver is considered to be of primary importance in the formation of plasma proteins (19, 21), this damage may be the cause of the fall in serum protein level. But quality and quantity of ingested protein also influence serum protein production (21). The series of serum protein determinations in groups II and III (fig. 5, 6) indicate that the serum protein level was directly and promptly influenced by nitrogen intake.

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[3] The vitamin A determinations were done by Lillian S. Bentley.

The results obtained with heated casein in these experiments are different from those previously described (1, 5) in which rats were used. In the rat, the digestibility of the heated casein was not markedly lowered, but the availability of the absorbed nitrogen was decreased. Supplementation of heated casein with lysine or histidine improved its biological value for rats. That the fraction of the heated casein nitrogen absorbed in these experiments with dogs was not as completely utilized as was that of the unheated casein can be inferred from the decrease in growth rate and lowering of serum protein levels which occurred in the animals fed large amounts of the heated protein. Thus the dogs in group III, which were fed heated casein at the 24 per cent level, might be expected to approximate the performance of their littermates fed the unheated casein at the 18 per cent level if the 34 per cent loss in digestion were the only disability inflicted. But neither growth nor serum protein levels bear out this conclusion (fig. 3 and 6). The serum protein and albumin levels shown by *dogs 15* and *17* were higher than those of *dogs 11* and *13*, but the positive control of the former dogs, *no. 16*, had also slightly higher levels than *dogs 12* and *14* which were fed the unheated diet containing 18 per cent casein. The globulin levels of all the dogs of this group fed the heated protein were about the same as those of their controls on the unheated diets, thus possibly indicating some failure of utilization of the absorbed heated nitrogen in albumin, but apparently not in globulin formation. This may mean that the amino acid fraction required for globulin formation was altered by heat more than was that required primarily for albumin formation. This is in agreement with the findings of Seeley (22), who reported that casein caused equal regeneration of albumin and globulin in depleted dogs, beef serum protein regenerated only albumin, but amigen, a casein hydrolysate, favored only globulin production. Seeley likewise found that depleted dogs excreted less urinary nitrogen than did normal animals, as was found to be the case in these experiments, all dogs on heated diets excreting less nitrogen in the urine, even on greater intakes and after allowance had been made for intestinal loss, than did those fed unheated protein (fig. 7, 8). The failure of casein acid hydrolysates fed intravenously to maintain weight and nitrogen balance in dogs, even when supplemented by cystine, as reported by Frost and Risser (12), may be in part at least due to the same type of heat damage manifested in whole casein. The fatty or vacuolized livers of their dogs, ascribed to sulfur amino-acid deficiency, were also seen in the dogs fed severely heated casein in these experiments.

The addition of l-lysine dihydrochloride to the heated diets, which had been found remarkably effective in improving the growth of rats (1, 5), was also measurably useful in counteracting the heat effect in the one dog in which it was tried for a long enough period. The increase in growth, serum protein levels and nitrogen retention was unmistakable. There was, however, no accompanying alleviation of the digestive loss of nitrogen, and the animal did not equal its positive control, *dog 16*, nor greatly exceed the performance of *dog 15*, except in globulin formation, fed the heated casein without lysine supplement. It may be that the amount of lysine given was insufficient.

The improvement obtained with 3 grams lactalbumin daily in *dog 13* was similar. This much lactalbumin provided about 0.3 gram lysine, and 0.2 gram cystine and methionine combined, and the net effect equalled that of 1 gram lysine in *dog 17*. Since this simultaneous addition of lysine and methionine and cystine and other amino acids even in small amounts appeared to be as effective as the larger intake of lysine, the failure of absorption of the heated protein could hardly have been due entirely to unavailability of lysine.

The depression of biological value of a protein by heat may result from either decrease in absorbability of usable amino acids or in the availability of the completely absorbed amino acids. According to Seegers and Mattill (23) liver proteins heated at 120° for 72 hours or hot-alcohol-extracted for 130 hours had markedly lowered digestibility for rats, but acid hydrolysates of these proteins had the same digestibility and biological value as the original dried liver proteins. A later study (24) of the digestion *in vitro* of liver and kidney proteins, fresh and hot-alcohol-extracted, revealed the loss of digestibility in some of these proteins but not in others, and also a loss of free amino nitrogen without loss of total nitrogen. This was interpreted to indicate the formation in the denatured proteins of new enzyme-resistant anhydride linkages between the ε-amino group of lysine and available hydroxyl groups. Presumably these abnormal linkages were destroyed by complete acid hydrolysis.

In the case of the heated casein fed to dogs in our experiment the evidence points to failure of digestion and absorption as the primary and in the less severely heated protein possibly the entire cause of the decreased biological value. Apparently any enzyme-resistant peptides produced by dry heat are not absorbable when fed to the dog, as they are when fed to the rat, with resultant increase in fecal, and decrease in urinary, nitrogen in the former species. The net loss of biological value of the heated protein is nearly the same in the two species. Murlin, Nasset and Marsh (4) as a result of experiments with human subjects, reported reduction in egg-replacement value in wheat breakfast cereals which had been subjected to high temperatures, about 200°, in the process of manufacture. The reduction was of the same order as that reported for similar products fed to rats (1) and was ascribed to losses in metabolism with no reduction in true digestibility.

Mecham and Olcott (25) found that dry heat caused several proteins, including casein, to become increasingly insoluble and resistant to digestion *in vitro* by pancreatin. These effects were measurable in proteins heated at 110° to 140°

and much exaggerated when temperatures from 150° to 203° were used. This parallels the markedly increased loss of digestibility for dogs of the casein heated at 200° as compared with that heated at 140° in our experiments.

SUMMARY

1. Young dogs were fed from weaning to maturity on purified diets containing casein, unheated or heated at 140° or 200° for 15 or 30 minutes. The protein levels used were 18, 24 and 36 per cent of the diet.

2. The growth of the animals on the heated protein was inferior, particularly on that exposed to the higher temperature. Little advantage was seen in the use of the 24 per cent as compared with the 18 per cent heated diet and rapid deterioration occurred in one dog when 36 per cent heated casein was substituted for the 18 per cent diet.

3. The administration of 40 grams of 1-lysine dihydrochloride in 52 days to one dog caused slight but perceptible improvement in growth and serum protein level. A similar change occurred in another dog on the heated diet when 3 grams lactalbumin was given daily for two months.

4. The total serum protein and albumin levels, but not the globulin level, were progressively lowered in all the animals on the heated protein. Hemoglobin concentration was also decreased although less strikingly.

5. Total nitrogen intake was maintained equal in the paired animals on heated and unheated diets by force feeding. Nevertheless decreasing retention and eventual nitrogen loss occurred on the heated protein, with most of the excess excretion appearing in the feces. The apparent digestibility of the casein heated at 200° was 28, of that heated at 140°, 67, and of the unheated 91. True digestibility of the more severely heated casein in one case was found to be 30.

6. Running fits developed in the two dogs which had been treated with lysine or lactalbumin and in no others.

7. Yellow livers, apparently fatty, were found in all the dogs fed the 200° heated casein, and low heart and muscle creatine in one. These conditions point to possible methionine deficiency.

8. The differences between these observations and those seen previously in rats similarly fed indicate poorer capacity on the part of the dog for absorption of any abnormal amino acid or peptide forms present in the heated casein but similar net loss of biological value in the two species.

REFERENCES

1. Morgan, A. F. J. Biol. Chem. **90**: 771, 1931.
2. Chick, H., M. A. Boas-Fixsen, J. C. D. Hutchinson and H. M. Jackson. Biochem. J. **29**: 1712, 1935.
3. Fairbanks, B. W. and H. H. Mitchell. J. Agr. Research **51**: 1107, 1935.
4. Murlin, J. R., E. S. Nassett and M. E. Marsh. J. Nutrition **16**: 249, 1938.
5. Greaves, E. O., A. F. Morgan and M. K. Loveen. J. Nutrition **16**: 115, 1938.
6. Arnold, A. and C. A. Elvehjem. J. Am. Vet. Med. Assoc. **95**: 303, 1939.
7. Kingsley, G. R. J. Biol. Chem. **49**: 109, 1921.
8. Howe, P. E. J. Biol. Chem. **49**: 109, 1921.

9. Rose, W. C., O. M. Helmer and A. Chanutin. J. Biol. Chem. **75:** 543, 1927.
10. Davies, A. W. Biochem. J. **27:** 1770, 1933.
11. Yudkin, S. Biochem. J. **35:** 551, 1941.
12. Frost, D. V. and W. C. Risser. J. Nutrition **32:** 361, 1946.
13. Robscheit-Robbins, F. S., L. L. Miller and G. H. Whipple. J. Exp. Med. **85:** 243, 1947.
14. Morgan, A. F. and M. Groody. J. Am. Vet. Med. Assoc. **105:** 406, 1944, **108:** 179, 1946.
15. Popper, H. and F. Steigman. J. Am. Med. Assoc. **123:** 1108, 1943.
16. Ralli, E. P., E. Bauman and L. B. Roberts. J. Clin. Invest. **20:** 709, 1941.
17. Popper, H., F. Steigman, K. A. Meyer and S. Zevin. Arch. Internal. Med. **72:** 439, 1943.
18. Popper, H., F. Steigman and H. A. Dyniewiscz. Proc. Soc. Exp. Biol. Med. **50:** 266, 1942.
19. Weech, A. A. and E. Goettsch. Bull. Johns Hopkins Hosp. **63:** 154, 1938.
20. Elman, R. and C. J. Heifetz. J. Exp. Med. **73:** 417, 1943.
21. Madden, S. C. and G. H. Whipple. Physiol. Revs. **20:** 1194, 1940.
22. Seeley, R. D. This Journal **144:** 369, 1945.
23. Seegers, W. H. and H. A. Mattill. J. Biol. Chem. **110:** 531, 1935.
24. Seegers, W. H. and H. A. Mattill. J. Nutrition **10:** 271, 1935.
25. Mecham, D. K. and H. S. Olcott. Ind. Eng. Chem. **39:** 1023, 1947.

OBSERVATIONS CONCERNING THE EFFECTS OF (1) SODIUM SALICYLATE AND (2) SODIUM SALICYLATE AND GLYCINE UPON THE PRODUCTION AND EXCRETION OF URIC ACID AND ALLANTOIN IN THE RAT[1]

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Salicylate is known to possess the property of increasing the urinary excretion of uric acid in both animal and man but the mechanism by which this is accomplished is not known.

Rockwood (1) believed that the drug acted to prevent the destruction of uric acid in the body, hence its increased excretion. Fine and Chace (2) however observed that concomitant with urinary increase in uric acid after the ingestion of salicylate, a decrease in blood uric acid occurred. Other workers (3, 6) have confirmed their findings, thus suggesting that the initial or primary site of salicylate action was probably renal.

Quick (7) believed that salicylic acid stimulated the renal excretion of uric acid. Gray and Grabfield (8) believed that denervation of the dog's kidney prevented the usual increase in urinary uric acid after the administration of salicylate. Talbott (9), employing various clearance techniques in his studies, was unable to find any change in the glomerular filtration rate of patients receiving salicylate, despite the fact that their urine did contain more uric acid. He concluded that salicylate probably interfered with the tubular reabsorption of uric acid. On the other hand, Klemperer and Bauer (10) found that the maximum urinary concentration of uric acid in subjects receiving salicylate was no different than that of control subjects. Because of this finding, they concluded that the increase in urinary concentration of uric acid following salicylate was not due to a change in renal tubular reabsorption.

In the present communication, results of studies concerning *a*) the renal hemodynamics and *b*) the uric acid and allantoin in the blood and urine of rats receiving sodium salicylate alone or combined with glycine are given. The evidence obtained strongly suggests that salicylate acts to increase the excretion of uric acid exactly as glycine was found to do (11), namely, by interfering with the renal tubular mechanism concerned with the excretion of uric acid.

METHODS

Both the acute and chronic experiments were done on male albino rats, weighing 275–350 grams, which were maintained on Purina Dog Chow.

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[1] Aided by a grant from the Public Health Service.

Uric acid, creatinine and hippurate clearances were obtained on rats according to our previously described methods (11, 12).[2]

RESULTS

1. *Acute effect of sodium salicylate upon the uric acid output and upon the renal hemodynamics of the normal rat.* Twenty-three rats were used in this study. Uric acid, creatinine and hippurate clearances were obtained on 11 control rats and on 12 rats which received 10 mgm. of sodium salicylate in 10 cc. of H_2O per 100 grams of body weight by mouth at the beginning of the clearance study (10 A.M.) and 5 mgm. of sodium salicylate in 5 cc. of H_2O every hour during the collection period of five hours.

Sodium salicylate in the dosage used in these experiments increased the uric acid excretion promptly and markedly in the rat. Thus the average excretion of uric acid of the control rats was 0.147 mgm. per hour (see table 1 A) and that of the 12 rats receiving salicylate was 0.294 mgm. per hour (see table 1 B). This increase of approximately 100 per cent in the excretion of uric acid however was not preceded by any significant rise in the blood uric acid (compare table 1 A and B). Furthermore, this increased excretion of uric acid after salicylate was not accompanied by any significant change in the urine volume, rate of glomerular filtration or in rate of renal plasma flow (the latter two functions were measured by the creatinine and hippurate clearances respectively). The average control creatinine and hippurate clearances were 35.9 and 141.0 cc. per hour per 100 grams of body weight respectively (see table 1 A). After the administration of salicylate, these same clearances were 33.2 and 152.0 cc. respectively (see table 1 B).

2. *Acute effect of sodium salicylate upon the blood and urinary allatoin concentration of the normal rat.* Twelve rats were used. Control determinations of the allantoin excretion per hour over a two-hour period were made on six of these rats. Similar determinations were made on the remaining six rats except that each received the same quantity of salicylate as the rats described above.

In these experiments, the administration of salicylate was not found to have any effect either upon the blood allantoin or upon the amount of allantoin excreted in the urine. The average blood allantoin (1.63 mgm. per 100 cc.) of the six control rats fell approximately 11 per cent during the clearance period, during which time the average amount of allantoin excreted was 2.16 mgm. per hour.

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[2] In respect to our method of determining the hippurate clearance which recently was misunderstood by Friedman, *et al.* (13), it should be stressed that two blood samples taken at the beginning and end of the clearance period were analysed for hippurate. The average of these two values was then multiplied by a correction factor (0.715). The use of this factor was found (after preliminary determinations of the plasma concentration of hippurate) to correct for the changing rate of plasma hippurate decline occurring during the first and second hours of the clearance. It cannot be stressed too much that a hippurate clearance based upon a single plasma analysis is completely unreliable. Our rats received 100 mgm. of hippurate subcutaneously which represented a dosage of approximately 30–35 mgm. per 100 grams of weight. The plasma concentration of hippurate during the clearance after such a dose rarely exceeded 4 mgm. per 100 cc. Allantoin in the blood and urine of rats was determined according to the method of Christman, Fosterer and Esterer (14).

TABLE 1. ACUTE EFFECT OF SODIUM SALICYLATE UPON THE URIC ACID CONTENT OF THE NORMAL RAT'S BLOOD AND URINE, AND UPON ITS RENAL HEMODYNAMICS

RAT	BLOOD URIC ACID DURING CLEARANCE[1]			U.F.	C.C.	H.C.	U.A.	U.A.C.
	10:00	12:00	3:00					
A. Control rats								
52	1.20	1.50	1.50	1.3	38.6	212.0	0.156	14.8
72	0.95	1.00	1.20	1.2	39.4	139.0	0.163	15.1
54	0.92	1.40	1.20	1.3	37.6	149.0	0.158	12.4
73	1.40	1.40	1.50	0.8	34.6	139.0	0.163	—
42	0.81	0.95	0.92	1.1	44.5	118.0	0.161	15.3
43	1.00	1.30	1.60	1.1	45.5	122.0	0.132	9.5
89	1.20	1.80	1.40	1.6	34.5	108.0	0.139	9.6
21	1.10	1.50	1.50	1.49	34.6	146.0	0.137	10.1
92	1.92	1.10	1.90	1.10	34.8	—	0.122	11.9
53	0.80	1.20	0.84	1.17	24.3	158.0	0.151	15.9
29	0.88	0.88	0.72	1.44	28.5	115.0	0.139	16.7
Av.......	1.11	1.28	1.30	1.24	35.9	141.0	0.147	13.1
B. Rats given sodium salicylate								
39	1.30	1.10	1.30	1.44	27.9	216.0	0.330	26.9
41	1.10	1.00	1.00	1.46	35.5	159.0	0.246	23.9
68	1.30	1.20	1.30	1.34	30.2	135.0	0.326	25.8
32	1.20	1.00	1.30	1.47	33.4	125.0	0.422	36.4
66	1.00	1.70	—	1.04	26.5	177.0	0.284	17.8
72	1.18	1.30	1.40	1.18	37.7	123.0	0.236	19.2
57	0.95	1.20	1.20	1.15	29.4	127.0	0.284	25.4
44	1.30	1.40	1.20	1.42	28.5	140.0	0.286	22.0
69	1.20	1.30	1.40	1.31	33.0	120.0	0.254	19.5
43	1.00	1.00	1.50	1.32	43.0	171.0	0.278	22.0
22	1.00	1.00	0.98	1.24	31.0	181.0	0.314	31.4
54	1.10	1.40	1.30	1.48	41.7	148.0	0.272	21.7
Av.......	1.14	1.22	1.26	1.32	33.2	152.0	0.294	24.3

[1] The blood uric acid samples were taken at the beginning, middle and end of the collection period.

U.F. equals urine flow in cc. per hour per 100 grams of body weight.
C.C. equals creatinine clearance in cc. per hour per 100 grams of body weight.
H.C. equals hippurate clearance in cc. per hour per 100 grams of body weight.
U.A. equals mgm. of uric acid excreted in urine per hour.
U.A.C. equals uric acid clearance in cc. per hour per rat.

The average blood allantoin (1.75 mgm. per 100 cc.) of the six rats which received salicylate remained approximately unchanged (1.77 mgm. per 100 cc.) during the clearance period. The average amount of allantoin excreted was 2.05 mgm. per hour. On the basis of these results, it seems likely that the ingestion of salicylate had no significant effect either upon the blood or upon the renal excretion of allantoin.

3. *Acute effect of sodium salicylate and glycine upon the blood and urinary uric acid of the normal rat.* Uric acid clearances were done upon nine rats which not only received the usual amount of sodium salicylate but also 50 mgm. of glycine by mouth at the beginning of the collection period and five mgm. every hour of the clearance determination.

The combined administration of salicylate and glycine was followed by an increase in the amount of uric acid excreted without a preceding or concomitant abnormal increase in the blood uric acid. However, the average uric acid excretion of the nine rats was 0.318 mgm. per hour which was only about eight per cent higher than the excretory rate of rats receiving only salicylates (see table 1 B). Moreover the average uric acid clearance in this series was only 17.7 cc. per hour as compared to 24.3 cc. of rats receiving salicylate alone. Whether this lowered clearance represents an interference by glycine or whether it resulted because of the reduced urine flow is unknown. It would appear then that little if any augmentation was obtained in the excretion of uric acid by the combined administration of glycine and salicylate.

4. *Effect of sodium salicylate upon the blood uric acid and allantoin of nephrectomized rats.* Fourteen rats were nephrectomized after control determinations of blood uric acid and allantoin had been made. Five of these rats received 30 mgm. of sodium salicylate in three divided doses in the 24-hour period following nephrectomy. Blood samples were obtained again from each of the rats, 24 hours after nephrectomy, and analyzed for uric acid and allantoin.

The administration of sodium salicylate had no effect upon the blood uric acid of nephrectomized rats. The average blood uric acid of the nine control rats was 1.34 mgm. per 100 cc. before and 1.49 mgm. per 100 cc. 24 hours after nephrectomy. The blood uric acid of the five rats which received salicylate was 1.40 mgm. per 100 cc. before and 1.26 mgm. 24 hours after nephrectomy.

Salicylate moreover was found to have no effect upon the blood allantoin concentration of nephrectomized rats. Thus the average blood allantoin of the control rats was 2.10 mgm. per 100 cc. before and 37.37 mgm. per 100 cc. 24 hours after nephrectomy. The average blood allantoin concentration of the five rats which received salicylate was 35.6 mgm. per 100 cc., 24 hours after nephrectomy.

5. *Chronic effect of sodium salicylate upon the blood and urinary uric acid content of the normal rat.* Six rats were caged individually and the daily urinary output was recorded for seven days. Determinations also of the uric acid in the urine of these rats were made every other day during the same control period. The average daily uric acid output for a week's period was then calculated by multiplying the average daily volume of urine in cc. by the average uric acid concentration of the three urine samples analyzed. Likewise the blood uric acid of these rats was determined every other day and the average daily blood uric acid for a week's period was calculated by averaging the uric acid content of three blood samples analyzed during the control perid.

After these control studies had been completed, each rat received 30 mgm. of sodium salicylate a day (in three divided doses) by stomach tube. Determinations of urinary and blood uric acid were obtained three times a week and averaged exactly as in the control period. The experiment was continued for 42 days after the control period.

Although the experiment began with six rats only three remained at the end of the sixth week, at which time the feeding of sodium salicylate was discontinued. The other three rats died following accidental perforation of their esophagus by the stomach tube during the first two weeks of the experiment.

As was expected (see table 2), the oral administration of salicylate was followed by a prompt increase in the amount of uric acid excreted per day. The average daily excretion of uric acid was 3.04 mgm. before and 4.43 mgm. (an increase of 45.7 per cent) during the first week of salicylate ingestion. As table 2 indicates the average daily uric acid excretion in subsequent weeks continued to be greater than that during the control period although never as large as that obtained during the first week of salicylate feeding. During the sixth week the average daily uric acid excretion of the remaining three rats was 3.72 mgm. (an increase of 22.4 per cent above the control value).

TABLE 2. CHRONIC EFFECT OF SODIUM SALICYLATE UPON THE URIC ACID CONTENT OF THE NORMAL RAT'S BLOOD AND URINE

DAYS	NUMBER OF RATS	BLOOD URIC ACID (MGM/100 CC.)		URINE FLOW (CC/DAY)		URIC ACID EXCRETED (MGM/DAY)	
		Average	Range	Average	Range	Average	Range
1- 7[1]	6	1.22	(1.1–1.5)	9.1	(6.5–11.0)	3.04	(2.5 –3.74)
7–14	6	1.30	(1.2–1.4)	14.3	(12.7–17.0)	4.43	(3.66–4.90)
14–21	4	0.90	(0.8–1.0)	14.1	(11.6–20.2)	3.94	(3.34–4.20)
21–28	3	0.91	(0.8–1.1)	18.1	(15.4–20.7)	4.02	(3.82–4.29)
28–35	3	0.53	(0.5–0.6)	18.8	(17.6–21.2)	3.88	(3.77–4.09)
35–42	3	0.53	(0.5–0.6)	20.9	(17.0–26.1)	4.24	(4.02–4.60)
42–49	3	0.50	(0.5–0.5)	19.2	(12.6–26.9)	3.72	(3.17–4.07)

[1] Control period of seven days during which no salicylate was given.

The average blood uric acid of the chronic rats was 1.22 mgm. per 100 cc. during the control period, decreased to 0.90 mgm. at the second week of salicylate administration and apparently became fixed at approximately 0.5 mgm. per 100 cc. from the fourth week on.

Coincident with the marked increase in uric acid excretion, the average daily urine volume increased from the control value of 9.1 cc. to 14.3 cc. during the first week of the experiment (see table 2). This increase in urine volume continued throughout the entire experiment.

DISCUSSION

Our experimental observations in common with those of previous investigators (1–5) clearly indicated that salicylate effected a marked and very rapid increase in the excretion of uric acid. The inability of salicylate to cause a change in the blood uric acid preceding this increased urinary excretion either in the acute or chronic experiments suggested that the increase was not the result of a changed or accelerated purine metabolism. Furthermore, the inability of salicylate to effect any change in the renal excretion of allantoin (which also is a product of the rat's purine metabolism) added additional evidence to suggest that salicylate did not act upon the mechanisms primarily involved in purine metabolism. Finally, the inability of administered salicylate to produce any change in either the blood uric acid or allantoin concentration of nephrectomized rats left little doubt that the increased excretion of uric acid after salicylate was due solely to some sort of renal phenomenon.

The absence of any significant change in the creatinine and hippurate clearances or in the excretion of allantoin following the ingestion of salicylates indicated that the increase in urinary uric acid following the latter could not have been due to either an increase in renal blood flow or in rate of glomerular filtration. Thus it would seem most likely that salicylate, as Talbott (9) has postulated, acts to influence the renal tubular mechanism involved in the excretion of uric acid. In this regard, it acts similarly to glycine which also has been found (11) to interfere with the excretion of uric acid. It is quite possible that both of these substances act upon the same tubular mechanism. The inability of both substances when given together to increase the uric acid output significantly above that following the ingestion of salicylate alone might be due to the possibility that the latter drug, in the dosage used, effected a physiological maximum of tubular interference in this regard.

Whether this tubular mechanism is influenced by salicylate only when the renal innervation is intact, as stated by Gray and Grabfield (8), is not yet determined. Unfortunately, the control studies of these authors indicated an increase in not only the excretion of uric acid but also of allantoin and urinary nitrogen, following the ingestion of salicylate. These last two increases are very difficult to understand in view of the fact that the creatinine clearance of their animals remained unaffected by the administration of salicylates. Since the allantoin clearance has been found also to be a measure of glomerular filtration in both the rat and dog (15) it seems strange that this clearance should increase and the creatinine clearance (its exact counterpart) should remain unchanged. Likewise, the unexplained increase of urinary nitrogen (2–4 grams), after salicylate with no change in the creatinine clearance again, throws some doubt upon the accuracy of their control studies.

CONCLUSIONS

1. Sodium salicylate was found to effect an immediate increase in the urinary excretion of uric acid of the normal rat without a preceding or concomitant change in the blood uric acid, the renal plasma flow or the glomerular filtration rate.

2. Sodium salicylate was found to exert no effect either upon the blood or urinary allantoin content of the normal rat.

3. Glycine was not found to augment significantly the effect of sodium salicylate in increasing the excretion of uric acid.

4. Sodium salicylate was unable to cause any significant change in the blood uric acid or allantoin of the nephrectomized rat.

5. The chronic administration of sodium salicylate was found to cause an immediate and continued increase in uric acid excretion. The blood uric acid of such treated animals progressively fell to approximately one third of its control value.

6. It was concluded that salicylate acts to increase the output of uric acid by interfering with the tubular mechanism concerned with the excretion of uric acid.

REFERENCES

1. Rockwood, E. W. This Journal **25,** 34, 1909–10.
2. Fine, M. S. and A. F. Chace. J. Biol. Chem. **21,** 371, 1915.
3. Denis, W. J. Pharm. & Exp. Therap. **7,** 255, 1915.
4. Benedict, S. R. Harvey Lectures, Ser. XI, 346, 1915–16.
5. Rose, W. C. Physiol. Rev. **3,** 544, 1923.
6. Grabfield, G. P. and E. Knapp. J. Pharm. & Exp. Therap. **32,** 341, 1928.
7. Quick, A. J. J. Biol. Chem. **101,** 475, 1933.
8. Gray, M. G. and G. P. Grabfield. J. Pharm. & Exp. Therap. **52,** 383, 1934.
9. Talbott, J. H. Gout, Oxford Medicine. New York, Oxford University Press. **4,** 79, 1940.
10. Klemperer, F. and W. Bauer. J. Clin. Invest. **23,** 950, 1944.
11. Friedman, M. J. Clin. Invest. **26,** 815, 1947.
12. Friedman, M. This Journal **148,** 387, 1947.
13. Friedman, S. M., J. R. Polley, and C. L. Friedman. This Journal **150,** 340, 1947.
14. Christman, A. A., P. W. Foster, and M. B. Esterer. J. Biol. Chem. **155,** 161, 1944.
15. Friedman, M. Clearance of allantoin in the rat and dog as a measure of glomerular filtration rates. To be published.

EFFECT OF FOLIC ACID AND LIVER EXTRACT ON SERUM AND RED CELL CHOLINESTERASE ACTIVITY

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The irreversible inactivation of cholinesterase (ChE) by di-isopropylfluorophosphate (DFP) has made possible numerous studies of the rates of regeneration of red cell and plasma ChE. It has been shown, in animals treated with DFP, that red cell ChE is restored at a rate commensurate with erythropoiesis and that plasma ChE restoration proceeds at a rate closely similar to that of the regeneration of other plasma proteins (1, 2). It is probable that the regeneration rate of a given tissue ChE after DFP reflects the protein production rate characteristic of that tissue.

Recent reports (3–5) state that liver extract and folic acid are effective in the treatment of anemia by virtue of an alleged capacity for enhancing serum ChE activity both *in vitro* and *in vivo*. This increase in serum ChE activity is said to occur both in normal animals and in those with reduced ChE levels. The experiments reported herein were prompted by interest in the alleged role of ChE in erythropoiesis, the fundamental interest which attaches to these observations with respect to protein synthesis in general, the mechanism of action of drugs—in either activating a specific protein, viz., ChE or in accelerating its restoration in the body—and the possible therapeutic value of folic acid or liver extract in poisoning by anti-cholinesterase agents.

METHODS

1. *In vitro experiments.* Blood plasma samples were obtained from normal humans, normal dogs, and dogs chronically poisoned with DFP. To these were added varying concentrations of folic acid[1] or liver extract and incubation was carried out for 2½ hours at 37°C. in a constant temperature bath. Plasma ChE activity was determined manometrically as previously described in application to a study of human liver disease (6).

2. *In vivo experiments.* Four normal female dogs were used; they were maintained in good health on a diet constant in composition prior to and during the study. Determinations were made of plasma ChE activity, erythrocyte counts, hemoglobin percentages and reticulocyte percentages. The Haden-Hausser hemoglobinometer was used for determining hemoglobin percentages; reticulocyte counts were made on blood smears stained with brilliant cresyl blue and counterstained with Wright's stain.

The solutions of folic acid, acetylcholine bromide and physostigmine were made in distilled water; the acetylcholine solution was buffered with sodium phosphate to reduce irritation at the site of injection. Armour's 'Liver Liquid Parenteral' was used; it contains 15 U.S.P. units in 1 cc.

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[1] The folic acid used was supplied by Lederle Laboratories.

RESULTS

1. *In vitro.* The incubation of normal human plasma with relatively large concentrations of folic acid or liver extract produced no significant change in ChE activity from control figures. An example of such results appears in table 1.

TABLE 1. EFFECT OF FOLIC ACID AND LIVER EXTRACT ON CHE ACTIVITY OF NORMAL HUMAN PLASMA

INCUBATION MIXTURE	ChE ACTIVITY (MM³ CO₂/0.1 CC/30 MIN.)
1.0 cc. serum	217
1.0 cc. serum +0.2 mgm. FA	219
1.0 cc. serum +0.08 u. LE	209
1.0 cc. serum +0.75 u. LE	217

TABLE 2. EFFECT OF FOLIC ACID AND LIVER EXTRACT ON THE PLASMA CHE ACTIVITY OF DOGS TREATED WITH DFP

DOG	INCUBATION MIXTURE	ChE ACTIVITY (MM³ CO₂/0.1 CC/30 MIN.)
1	1.0 cc. plasma	30
	1.0 cc. plasma + 0.2 mgm. FA	28
	1.0 cc. plasma + 0.5 mgm. FA	33
	1.0 cc. plasma + 1.0 mgm. FA	31
2	1.0 cc. plasma	67
	1.0 cc. plasma + 10.0 mgm. FA	68
	1.0 cc. plasma + 2.5 u LE	78
2	1.0 cc. plasma	68
	1.0 cc. plasma + 10 mgm. FA	67
	1.0 cc. plasma + 2.5 u LE	67
2	1.0 cc. plasma	79
	1.0 cc. plasma + 0.75 u LE	79
	1.0 cc. plasma + 0.16 u LE	78
3	1.0 cc. plasma	37
	1.0 cc. plasma + 0.2 mgm. FA	39
	1.0 cc. plasma + 0.75 u LE	35

Plasma samples from 3 dogs chronically poisoned with DFP, possessing 40 per cent or less of their normal (pre-DFP) ChE activity, were similarly incubated with folic acid and liver extract in wide ranges of concentration. In no instance did these drugs produce a significant change in the ChE activity. These results are shown in table 2.

2. *In vivo.* In an attempt to produce a macrocytic anemia and a lowering of plasma ChE activity (4), two dogs were given daily subcutaneous injections of 3.0 mgm. of acetylcholine bromide and 1.0 mgm. of physostigmine salicylate for a

period of 61 days; for the same period two dogs were kept as untreated controls. In neither of the two treated dogs were there any significant changes in plasma ChE activity, erythrocyte counts, reticulocyte percentages and hemoglobin percentages. In addition, there was no appreciable difference between the data obtained on these dogs and those obtained on the control animals.

During the subsequent 25-day period the acetylcholine and physostigmine injections were continued; in addition, although no anemia had been produced, one of the experimental animals and one of the control animals received a daily intramuscular injection of two units of liver extract. Throughout this 25-day period no significant changes were noted.

TABLE 3. EFFECT OF DRUGS ON BLOOD CONSTITUENTS

DOG	EXPERIMENTAL PERIOD	DRUGS	RED CELLS			HEMOGLOBIN	RETICULOCYTES	ESTERASE ACTIVITY (MM^{3}CO$_2$/0.1 CC/30 MIN.)	
			Detn.	Millions Av.	Millions Extremes			Av.	Extremes
	days					*grams %*	*per cent*		
1	13	none	4	5.37	5.15–5.81	12.5	0.1	128	125–130
	61	AcCh, Phys	13	5.82	5.14–6.81	12	0.1	118	101–141
	25	AcCh, Phys LE	3	6.05	5.58–6.49	13	0.2	108	84–116
	12	AcCh, Phys FA	2	5.82	5.52–6.12	14.5	0.1	109	105–113
2	13	none	4	5.57	5.14–6.01	13	0.1	77	72– 78
	61	AcCh, Phys	13	6.09	5.16–6.44	13	0.2	75	55– 91
	25	AcCh, Phys	3	5.75	5.17–6.19	14	0.1	71	66– 75
	12	AcCh, Phys FA	2	6.06		14	0.2	71	70– 72
3	13	none	4	6.69	6.10–7.38	15	0.2	81	78– 82
	61	none	13	6.83	5.82–7.48	14	0.2	74	59– 90
	25	none	3	5.89	4.95–6.70	14	0.2	78	73– 82
	12	none	2	6.50	5.99–7.00	14	0.1	74	72– 75
4	13	none	4	6.56	5.84–7.33	15	0.2	73	71– 74
	61	none	13	6.84	5.89–7.30	14.5	0.2	74	61– 89
	25	LE	3	6.74	6.62–6.89	14.5	0.1	81	74– 84
	12	none	2	6.57	6.32–6.82	14.5	0.1	78	76– 80

In order to test the possible effect of folic acid on the blood constituents the experimental dogs were placed on a final 12-day period during which they received daily an intramuscular injection of two mgm. of folic acid in addition to their usual acetylcholine and physostigmine. This procedure also proved fruitless; no significant changes were noted in any of the collected data.

The combined data on the four dogs for these experimental periods are shown in table 3. Although there was considerable variation in the red cell counts from day to day, no trend toward anemia was discernible. The esterase data likewise showed a variability over the three-month period but no trends were established.

A second series of experiments was carried out to test the validity of the thesis that folic acid and liver extract hastened the generation of plasma ChE. In this series the regeneration of plasma ChE was followed after the enzyme had been reduced to low levels by doses of DFP. Of the four dogs given DFP, two were allowed to continue without further treatment, one was given a daily injection of two mgm. of folic acid, and one was given a daily injection of two units of liver extract. The contour and time course of the recovery curves of plasma ChE in the dogs receiving liver extract and folic acid did not differ from those of the control dogs. The curves are shown in figure 1.

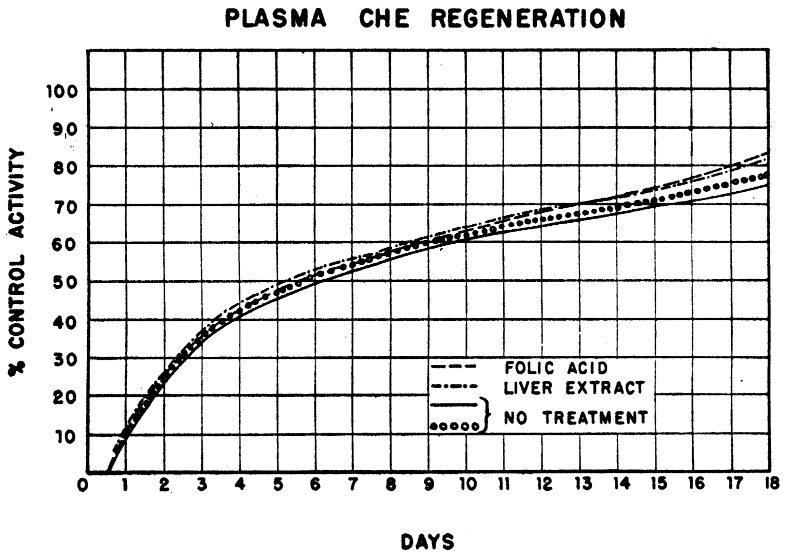

Fig. 1

DISCUSSION

Although the experiments are limited in number, these results do not support the idea that the ChE activity of both normal and ChE-depleted plasma may be affected *in vitro* by either folic acid or liver extract. Indeed, it seemed unlikely that a protein enzyme could be generated from precursors by incubation, but the possibility remained that folic acid or liver extract might act as ChE activators. This possibility has been discounted in the present experiments.

That the plasma ChE activity of normal dogs and of dogs with plasma ChE severely depleted by DFP may be influenced by parenteral administration of liver extract and folic acid is likewise not supported by the present experiments. Further study may be necessary to resolve the discrepancy between these and other published observations (3–5), but it is doubtful that liver extract or folic acid will prove valuable therapeutically for augmenting ChE activity in cases of poisoning with anti-ChE compounds. The reason for the discrepancy between our results and others is not obvious, for differences in methods of ChE determination are not recognized as giving such widely divergent results, at least in serum and erythrocytes. The ChE activities which we encountered were such that they lay in a range of our method sufficiently sensitive to make impossible a failure to detect increases of the order claimed by previous workers as due to liver extract or folic acid. Further, this circumstance also prevented small fortuitous changes from appearing as large percentage changes. How these matters stand with the titrimetric procedure used by others in this particular problem is difficult to assess from the published data. Hence, we can offer no explanation for the discrepancy.

Considerable variation was noted in the erythrocyte counts of the control and experimental animals, but no trend toward anemia was discernible as a result of acetylcholine and physostigmine administration in doses reportedly effective in this respect. Although choline feeding (4) was not employed in these experiments, the consistently negative character of the results in the problem as a whole does not lead to the expectation that choline might produce anemia or reduction in ChE activity. In this respect, other investigators (7) have been unable to produce a macrocytic anemia in dogs fed choline or choline and fat.

These considerations do not support the hypothesis that the acetylcholine-ChE mechanism is concerned with erythropoiesis. Recently published results of other investigators (2) in patients with pernicious anemia have proven that ChE is not concerned with erythropoiesis. Indeed it is much more logical to expect that any increase in the plasma ChE activity in patients treated for pernicious anemia is a resultant of recovery and not the cause of it.

CONCLUSIONS

1. Liver extract and folic acid *in vitro* and *in vivo* do not increase plasma ChE activity in normal or plasma ChE-depleted dogs.
2. Parenterally administered acetylcholine and physostigmine do not induce erythrocyte or hemoglobin changes suggesting anemia in dogs.

REFERENCES

1. Koelle, G. B. and A. Gilman. J. Pharmacol. and Exper. Therap. **87:** 421, 1946.
2. Grob, D., J. L. Lilienthal, Jr., A. M. Harvey and B. F. Jones. Bull. Johns Hopkins Hosp. **81:** 217, 1947.
3. Davis, J. E. Science **104:** 37, 1946.
4. Davis, J. E. This Journal **147:** 404, 1946.
5. Davis, J. E. Proc. Soc. Exper. Biol. and Med. **63:** 287, 1946.
6. Wescoe, W. C., C. C. Hunt, W. F. Riker and I. C. Litt. This Journal **149:** 549, 1947.
7. Clarkson, M. F. and C. H. Best. Science **105:** 622, 1947.

BLOOD PRESSURE CHANGES IN RESPONSE TO ELECTRICAL AND CHEMICAL (ACETYL-BETA-METHYLCHOLINE) STIMULATION OF THE CEREBRAL CORTEX IN DOGS

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The cortical representation of autonomic functions has been the subject of a number of recent investigations, which may be roughly classified under the following categories: *a*) studies of the autonomic effects of electrical cortical stimulation; *b*) studies of the autonomic effects of well-defined ablations; *c*) the recording of action potentials from the brain surface during stimulation of the vagal nerve; *d*) neurohistological studies of the intracerebral pathways to establish the relations of the allocortex with the hypothalamic centres.

Most experiments can be classified under the first heading. Although they all yield evidence of a cortical component in the regulation of autonomic functions, the use of different experimental animals often makes it difficult to compare the results as regards the exact location of such cortical action. Also, the information gained in this way is far from being unequivocal. Thus, Hoff and Green (1), investigating the anterior and posterior sigmoid gyri in cats under ether anesthesia, observed an elevation of blood pressure in response to electrical stimulation of this area, together with a dilatation of the pupils. This was confirmed in later experiments (2), in which, moreover, a simultaneous diminution in kidney volume and an increase in limb volume could be recorded, suggesting that a shift of blood from the visceral to the muscular bed is the underlying mechanism for the cortically induced blood pressure rise. Similar blood pressure responses were obtained by Crouch and Thompson (3) from the anterior sigmoid in cats (Dial, nembutal, ether anaesthesia). In dogs, however, they observed mainly a fall in blood pressure, while Spiegel and Hunsicker (4) obtained the blood pressure fall also in cats (ether anaesthesia). An earlier publication by Dusser de Barenne and Kleinknecht (5) described a fall in blood pressure upon stimulation of the motor cortex in cats, dogs and rabbits, while in 1942 Siao-Hsi *et al.* (6) again could obtain only depressor effects following stimulation of this area in dogs under ether-chloralose anaesthesia. Also, in further contrast to Hoff and Green's observations, they found the kidney volume increased. Bucy and Case (7), in a study of the cortical innervation of respiratory movements, mention rather inconstant blood pressure changes. One may conclude, while accepting the evidence of a vasomotor function of the sigmoid gyri, that we have no reliable information as to its exact character.

In this study concerned with the vasomotor effects of cortical stimulation, mention should be made also of blood pressure effects obtained from areas other than the sigmoid gyri. Hoff and Green (1) reported the presence of depressor points, diffusely scattered over the convexity of the hemispheres in cats, and of a pressor area in the superior precentral cortex in the macaque. Smith (8), who located an inhibitory effect on respiration in the lateral part of area 6 (cats, dogs) or 6b (monkey), stated that such inhibition was usually accompanied by a marked rise in blood pressure (ether anaesthesia). Bailey and Sweet (9) found the blood pressure noticeably raised upon stimulation of area 13 on the orbital brain surface of the macaque and of a similar region in the cat's brain (nembutal anaesthesia). This area had been previously described by Bailey and Bremer (10) as the sensory cortical end station of vagal nerve impulses.

The discrepancies in vasomotor responses obtained by the different authors upon stimulation of identical areas on the brain surface may find their explanation in the variability of a number of factors. Of these, some are unrelated to the animal itself, such as the kind of anaesthesia used, the depth of anaesthesia, the type of stimulation applied, and the strength of stimulation. Other factors are directly related to the animal, such as species and age, the general physiological state of the animal, especially with regard to the activity of the gastro-intestinal tract, the initial blood pressure at the start of the experiment, and the excitatory state of the brain surface which may change considerably during the course of the experiment.

In this study, the usefulness of a different type of stimulus, the local application of acetyl-beta-methylcholine, has been investigated and compared with that of electrical stimulation.

METHOD

All dogs, of different race, age and sex, received their last feeding in the evening previous to the day of operation and experimentation. Anaesthesia was induced by intraperitoneal injection of Dial, 45 to 50 mgm. per kgm., usually administered in two or three separate doses before the operation in order to avoid too deep an anaesthesia. In two dogs Na-amytal, 50 mgm. per kgm., was used.

After ligation of both common carotids, the frontal, parietal and most of the occipital and temporal lobes were exposed on either the left or right side, while in the majority of cases, one eye was enucleated in order to provide a better approach to the orbital brain surface. Before the dura was opened, one common carotid was connected with a mercury manometer, a 7 per cent Na-citrate solution being used as mediator between blood and mercury column. From the moment the dura was opened, the brain surface was kept wet by application of warm Ringer's solution.

In coagulating the connections of pial vessels with the sagittal sinus, the use of a monopolar 'Highfrecator' needle proved to be of great advantage; for such purposes this simple instrument must be considered far superior to the generally used electrocoagulator of lower frequency, as supplied with electrosurgical units.

In seven dogs the cortex was stimulated faradically with bipolar copper wire electrodes 1 mm. apart, while the current was supplied by a 3-volt battery, connected with a Harvard inductorium, using a coil distance varying between 5 and 8 cm. Each stimulation lasted from 10 to 15 seconds. In four dogs the cortex was stimulated with a 2.5 per cent solution of acetyl-beta-methylcholine, applied by means of minute pieces of filter paper soaked with this solution. The duration of the application varied from 40 seconds to 2 minutes.

RESULTS

In six of the animals, anaesthetized with Dial, blood pressure effects were easily obtained from either the anterior or posterior sigmoid gyrus or from both. A 7–8 cm. coil-distance of the Harvard inductorium provided an appropriate stimulus to provoke the effect from a fairly limited area. Stronger stimuli often elicited vasomotor responses from a far larger region, extending laterally into the coronal and ectosylvian gyri. The latter responses were usually accompanied by strong movements of the limbs and trunk, sometimes resulting in vigorous movements of the entire body. Therefore it was decided to consider only the effects of the weaker stimuli, with a coil distance of 8 cm. These, as well as the responses to stronger stimuli, always demonstrated themselves as a fall in blood pressure (fig. 2). The following characteristics were observed. Usually the depressor effect amounted to 15 to 20 mm. Hg, while in case of a high initial blood pressure a greater drop might occasionally be observed, not exceeding 30 to 35 mm. Hg. The drop started abruptly after a latent period varying between 6 and 9 seconds. It reached its lowest point within six seconds, but would never remain there for more than 2 to 3 seconds. From there it returned to its former level, in such a way that the more this was approached, the slower the rise would be. The whole phenomenon lasted from 20 to 25 seconds and was not influenced noticeably by the duration of the stimulus. Often upon stimulation of the sigmoid gyri, very slight movements of one of the opposite limbs were observed, while stimulation of the frontal part of the gyrus compositus anterior elicited rhythmic movements of the jaw with great regularity but with a considerable latency. Also from the latter area, the respiration was inhibited, while from the motor area the respiratory movements were intensified and accelerated. The results of the experiments in *dogs I, V* and *VI* are recorded in drawings of the stimulated areas in figure 1.

In *dog VII* a different anaesthesia was used, i.e., Na-amytal 50 mgm. per kgm. In this animal the respiratory effects were as easily obtainable as in the previous dogs. For vasomotor responses, however, stronger stimuli had to be used (coil-distance 5 to 6 cm.), and responses were evoked from a much wider area. Also, although never exceeding a rise of 5 mm., an elevation of blood pressure was observed equally as often as a drop, the latter usually being more pronounced than the former. Altogether it showed the picture of a lowered excitability for vasomotor responses which, when obtained, were inconstant and not sharply limited to one area, as compared with dogs under Dial anaesthesia. Figure 1 illustrates the findings in this animal (*dog VII*), although the drawing should not be compared with those for the other animals, since the stimulation was stronger and the responses weaker.

In an attempt to find a more physiological stimulus than those hitherto used, several chemical compounds were applied to the brain surface during the course of two of these experiments. The following were used: adrenaline, ephedrine, strychnine, prostigmin, and acetyl-beta-methylcholine. Only acetyl-beta-methylcholine evoked a marked vasomotor response, and it was considered to be of importance to investigate the possibilities and characteristics of this substance as a cortical stimulant in the study of blood pressure regulation.

In three animals anaesthetized with Dial, and in a fourth one anaesthetized with Na-amytal, it was demonstrated that this drug, if applied in a concentration of ten per cent, produced a marked depressor effect from a wide area. Concentrations of 2.5 per cent would still yield a marked response, but the action became very closely limited to a small surface region. Concentrations lower than two per cent usually remained without effect in the investigated areas. Therefore in the following experiments a concentration of 2.5 per cent has been used without exception.

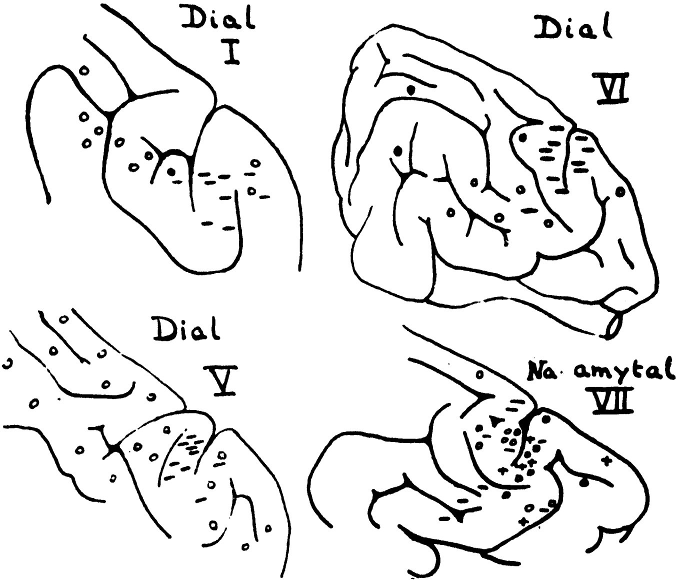

FIG. 1. BLOOD PRESSURE EFFECTS in response to faradic cortical stimulation. The drawings show the stimulated area around the cruciate sulcus in *dogs I, V* and *VI* (Dial-anaesthesia) and in *dog VII* (Na-amytal anaesthesia).

0 means: no blood pressure response; + means: elevation of blood pressure; — means: fall of blood pressure.

Two responsive areas were located: one in the posterior sigmoid gyrus near the midline (figs. 3 C, 4 C, 5 I, 7 C), the other in the anterior ectosylvian gyrus (figs. 6, 7 M). There was always a conspicuous drop in arterial pressure. The following characteristics could be observed: the depressor effect varied between 30 and 50 mm. Hg; the drop started abruptly, sometimes immediately preceded by an inconspicuous rise (fig. 6) after a latent period, varying between 25 to 40 seconds. During this drop, pronounced vasomotor waves of the third order[1]

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[1] The term 'third order waves' designates wave-like changes in the carotid blood pressure curve as compared to the 'first order' and 'second order' waves, respectively, representing the heart beat and the respiration. The first order waves are superimposed on the second order waves; the first and second order waves are superimposed on the third order waves.

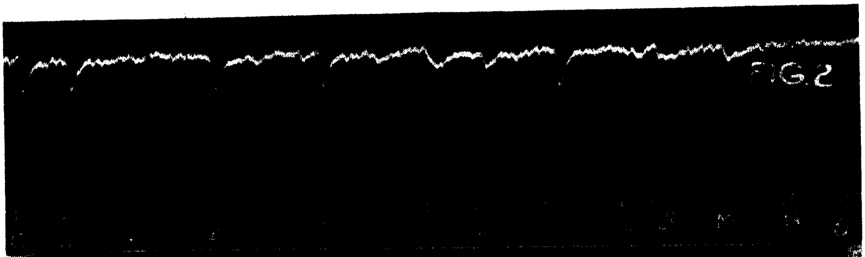


FIG. 2

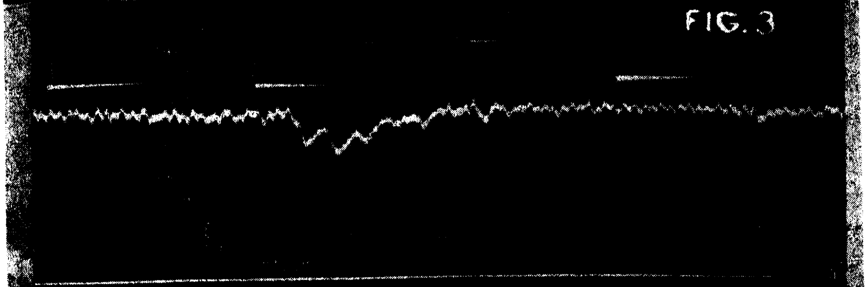


FIG. 3

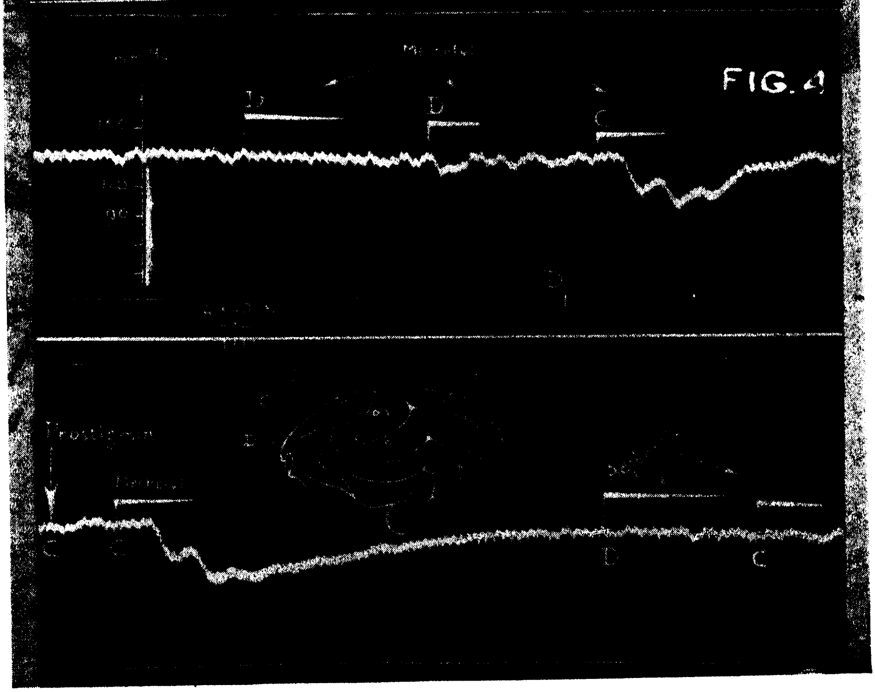
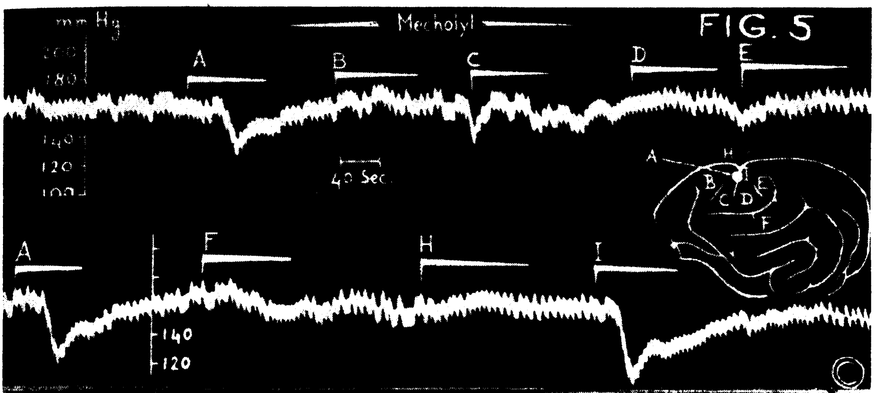


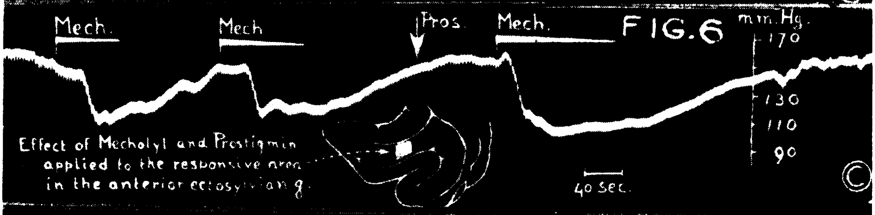
FIG. 4

FIG. 2. CHARACTERISTIC BLOOD PRESSURE RESPONSES to bipolar faradic stimulation in a Dial-anaesthetized dog (*dog III*).

FIG. 3. CHARACTERISTIC EFFECT with third order waves obtained by acetyl-beta-methylcholine application to responsive area C. Similar or electrical stimulation of areas E and F remains without effect (*dog A* same as in fig. 4).

FIG. 4. BLOOD PRESSURE CHANGES in response to local applications of acetyl-beta-methylcholine, prostigmin, prostigmin + acetyl-beta-methylcholine, adrenalin. The two blood pressure tracings are continuous. The capitals above the tracings correspond with the homonymous spots on the drawing of the cerebral cortex. Note that only *C* is responsive to acetyl-beta-methylcholine and that this response is considerably prolonged by simultaneous application of prostigmin, while adrenalin fails to elicit any blood pressure changes. The appearance of third order waves is clearly visible (*dog A*).





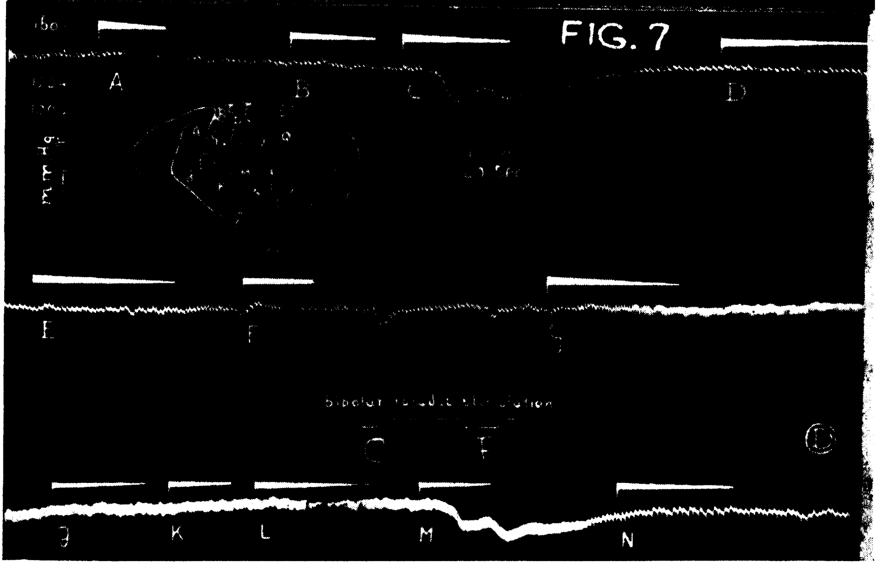


FIG. 5. UPPER AND LOWER TRACINGS ARE CONTINUOUS. The response from area *A* is probably due to stimulation of that part of the cortex which borders the cruciate sulcus posteriorly. A stronger effect was obtained from area I (*dog C*).

FIG. 6. STIMULATION OF ANTERIOR ECTOSYLVIAN GYRUS with acetyl-beta-methylcholine and prostigmin (*dog C*).

FIG. 7. THE TWO UPPER TRACINGS WERE CONTINUOUS. Responsive areas are found in *C* and *M*. Compare the effect of acetyl-beta-methylcholine stimulation with that of electrical stimulation of area *C* in the upper and middle tracing (*dog D*).

appeared with great constancy, at a rate of about one per 40 seconds. The lowest pressure level was reached in either the first or the second wave. Usually at least three waves could be recorded. The blood pressure then slowly returned to its former level. The whole phenomenon lasted from 2 to 3 minutes. A second application, rapidly succeeding the first one, produced a somewhat longer lasting effect, which may have been due to local cumulative action or to facilitation (fig. 6).

In all experiments the response was markedly restricted to a well circumscribed area. Immediately adjacent spots remained completely silent, even when repeatedly stimulated. Moreover, the responses were much more pronounced than those obtained by electrical stimulation of the same region.

In order to provide further proof for the cortical action of the drug, prostigmin salicylate powder was applied to the responsive spots. Although this substance did not evoke any vasomotor response, it was able to enhance and prolong conspicuously the action of consecutively applied acetyl-beta-methylcholine. On the other hand, locally applied pontocaine-HCl crystals could forestall and abolish the acetyl-beta-methylcholine effect.

It was further observed that, due to factors somehow related to the exposure, the brain surface showed considerable changes in its physiological state within one to one and one half hours after the dura had been opened, notwithstanding the fact that the cortex was kept wet continuously with Ringer's solution. Either all responses would disappear, or, as happened in one case, responses were obtained from almost any point on the exposed surface, previously silent, while freshly exposed parts still behaved normally.

One animal was anaesthetized with Na-amytal (Dog D). Here acetyl-beta-methylcholine showed the same effect, with the restriction that the blood pressure fell to a lesser degree, although the duration of the phenomenon remained the same, as compared with the other experiments.

DISCUSSION

When the vasomotor action of bipolar faradic stimulation was compared with that of local application of acetyl-beta-methylcholine the following facts were noted:

	FARADIC	ACETYL-BETA-METHYLCHOLINE
latent period	6 to 9 seconds	25 to 40 seconds
effect	drop in arterial pressure	drop in arterial pressure appearance of third order waves
character of drop	abrupt	abrupt
difference between highest and lowest level	15 to 20 mm. Hg	30 to 50 mm. Hg
site of action	fairly well restricted to circumscribed area	restricted to more sharply defined and smaller area
duration of the phenomenon	20 to 25 seconds	2 to 3 minutes

The difference in duration between the effects of both types of stimulation is of special interest, since continuation of the faradic stimulus did not appreciably prolong the effect. For that reason, the much longer-lasting response to acetyl-beta-methylcholine application cannot be attributed merely to a difference in duration of the stimulus; and although it is quite impossible to determine exactly the termination of the latter, it seems very likely that it involves a different mechanism than the former. This is further suggested by the appearance of vasomotor waves of the third order.

In this respect mention should be made of the work of Brenner and Merritt (11) and Forster and McCarter (12, 12a), who both draw attention to the similarity between the action of choline derivatives on the electrical activity of the cortex and the characteristic phenomena of cortical activity in epileptic patients. In 1937 Fiamberti (13) had produced generalized convulsions in man by means of intracisternal injections of acetylcholine. Williams (14) observed increased abnormal activity of the electroencephalogram upon intravenous injection of acetylcholine in epileptic patients.

Miller, Stavraky and Woonton (15) found no changes in the rabbits' electroencephalogram after injection of one per cent acetylcholine unless the animals were physostigminized. Brenner and Merritt (11) showed that this concentration was too low to be effective but that definite responses could be obtained with concentrations above 2.5 per cent. In cats, they also determined the liminal concentrations for acetyl-beta-methylcholine (1.5 per cent) and for carbamylcholine (0.3 per cent).

Local applications of acetylcholine to the cat's cortex (Chatfield and Dempsey, 16) were ineffective in a concentration of one per cent as shown in electrocorticographic studies of the somesthetic areas. In similar studies of the acoustic cortex in cats, Forster and McCarter (12) found acetylcholine highly effective in concentrations of 5 to 10 per cent. They made an interesting comparison with the cortical phenomena in epilepsy and their observation of self-continuing, intermittent, rapid electrical discharges ('Ach. discharges') may possibly explain the long-lasting effect of acetyl-beta-methylcholine in the experiments presented here.

From these data it would appear that acetylcholine, whether applied locally or administered systemically, increases the normally existing activity of the cortex, and one may apply this conclusion also to the action of acetyl-beta-methylcholine, since this drug differs pharmacologically from acetylcholine only in the respect that it is more slowly inactivated by cholinesterase.

Does this mean that the constantly obtained depressor effect reflects the true function of the cortical areas studied? It is thought that it does, but with the restriction that this function is conditioned by the actual status of the entire organism. In this respect one may regard as deciding factors *a*) the initial blood pressure, which in these experiments was above normal, probably because of the ligation of both common carotids; and *b*) activity or resting state of the gastro-intestinal tract. In these experiments, the animals had received no food for at least 12 hours; thus the gastro-intestinal tract was left fairly inactive, and favourable conditions were provided for a shift of blood from the muscular to the visceral bed.

It is, however, quite conceivable that under different conditions, as for instance a low initial blood pressure, the true regulatory function of the cortical centres would have demonstrated itself in a pressor response instead of the drop observed here.

Finally, a selective influence of the anaesthetic upon one phase of the cortical regulatory action must be considered, but should not be overemphasized as an explanation for experimental controversies. Up to the present, data concerning such influence have been rather contradictory, often obtained without due consideration of the many other varying factors which have been discussed above. In our experiments, the slight vasopressor effects observed in one animal anaesthetized with Na-amytal may possibly be regarded as the initial rise of an abortive drop, while their absence in Dial anaesthetized preparations certainly does not mean that the anaesthesia had abolished pressor responses, as these could still be obtained without difficulty from the hypothalamic centers.

SUMMARY

In experiments on 11 dogs, the usefulness of acetyl-beta-methylcholine as a cortical stimulant in the study of cortical blood pressure centers has been investigated and discussed.

Acetyl-beta-methylcholine proved to evoke a clear response from two circumscribed cortical areas: one in the motor cortex, the other in the anterior ectosylvian gyrus.

The response demonstrates itself by a drop in arterial pressure of 30–50 mm. Hg, with a latency of 30 to 40 seconds and a duration of 2 to 3 minutes, further characterized by the appearance of vasomotor waves of the third order.

Simultaneous application of prostigmin prolonged the effect considerably. The use of Na-amytal as a general anaesthetic instead of Dial affected only the stimulability of the cortex, but did not change the character of the blood pressure response.

Acetyl-beta-methylcholine may be considered to be a useful cortical stimulant, since it probably approaches the ideal physiological stimulus more than does electrical faradic stimulation.

REFERENCES

1. Hoff, Ebbe C. and Harold D. Green. This Journal **117**: 411, 1936.
2. Hoff, Ebbe C. and Harold D. Green. This Journal **118**: 641, 1937.
3. Grouch, Richard L. and J. Kenneth Thompson. J. of Nervous and Mental Disease **89**: 328, 1939.
4. Spiegel, E. A. and W. C. Hunsicker. J. Nervous Mental Disease **83**: 252, 1936.
5. Dusser de Barenne, J. G. and F. Kleinknecht. Z. Biol. **82**: 13, 1927.
6. Siao-Hsi Hsu, Kao Hwang and Ho-Nien Chu. This Journal **137**: 486, 1942.
7. Bucy, Paul C. and Theodore J. Case. J. Nervous Mental Disease **84**: 156, 1936.
8. Smith, Wilbur K. J. of Neurophys. **1**: 55, 1938.
9. Bailey, Percival and William H. Sweet. J. of Neurophysiol. **3**: 276, 1940.
10. Bailey, Percival and Frederique Bremer. J. of Neurophysiol. **1**: 405, 1938.

11. Brenner, C. and H. Houston Merritt. Arch. Neurol. and Psychiat. **48:** 382, 1942.
12. Forster, Frances M. and Robert H. McCarter. J. of Neuropathol. and Exp. Neurol. **5:** 24, 1946.
12a. Forster, F. M. Arch. Neurol. and Psychiat. **54:** 391, 1945.
13. Fiamberti, A. M. Riv. sper. freniat. **61:** 834, 1937.
14. Williams, D. J. Neurol and Psychiat. **4:** 32, 1941.
15. Miller, F. R., G. W. Stavraky and G. A. Woonton. J. Neurophysiol. **3:** 131, 1940.
16. Chatfield, P. O. and E. W. Dempsey. This Journal **135:** 633, 1942.

SPECIFIC SYMPATHOMIMETIC SUBSTANCE IN THE BRAIN[1]

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Knowledge of biologically active chemical constituents of the central nervous system, possessing a possible significance for cerebral function itself and for the principles of psychosomatics and psychochemistry, is very scant and fragmentary. Vasodepressant substances have been extracted from the brain by several investigators (1–6). Their chemical nature has not been identified, but it is certain that at least one of them is not identical with either histamine, choline or adenylic acid. Von Euler (6) believes that the 'substance P', described by him, is a protein. All authors found the highest concentrations of vasodepressant substances to be present in the basal ganglia (1, 2, 3, 5).

Other biologically active substances which appear to be closely related to, or even identical with, posterior pituitary hormonal fractions have been demonstrated in the tissue of the hypothalamus: the oxytocic principle (7, 8) the antidiuretic principle (8, 9) the blood-fat depressant principle ('lipoitrin,' 10) and the pigment hormone (11, 17).

The only statement regarding sympathomimetic vasopressor material in the brain has been made by von Euler (30), who found a very small amount of it in the brain of a calf and believes that it originates in vasomotor nerves, accompanying the brain vessels.

Numerous publications are concerned with the presence in the cerebrospinal fluid of biologically active material which is being attributed, in most instances, to secretory discharges from the posterior pituitary lobe. The following effects were found to be elicited either by the native cerebrospinal fluid or by extracts made from it: contractions of the uterus (12–15), stimulation of the intestines (16), inhibition of diuresis (15), expansion of melanophores (13, 17, 51) and elevation of the blood pressure (15, 16, 18, 19, 20, 26, 40). Stimulation of the hypothalamus is believed to be followed by an accumulation of pitressin or similar substance in the ventricular fluid (21). Most investigators agree that the highest concentrations of the respective active principles are present in the ventricular and cisternal fluid, while the lumbar fluid contains much smaller amounts, if any.

Page (20) described a vasopressor substance which he obtained through alcoholic extraction from ventricular and cisternal fluid of animals and man. He designated it as 'C.E.A.' (central excitatory agent).

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[1] This study was aided, in part, by a grant from the John and Mary R. Markle Foundation.

Using a slight modification (22) of Shaw's (23) colorimetric method for the determination of epinephrine and related compounds, the writer has found a substance with epinephrine-like chromogenic and adsorbability properties in the brains of rats (24)[2] and later in the brains of other mammals and humans, and in the human cerebrospinal fluid.

In the following, the results of quantitative and qualitative colorimetric and pharmacological studies of this substance are reported. The term 'encephalin' will be used to distinguish it from epinephrine and sympathin.

METHODS

Colorimetric determination of encephalin. Fresh brains were carefully freed of meninges and grossly discernible blood vessels, including the choroid plexus. Sections from various parts of the brain, weighing around 800 mgm., sections of the cerebellum, cord, choroid plexus and whole pituitary glands were ground with Ottawa sand in 10 per cent trichloroacetic acid, filtered, and the filtrates worked up as described elsewhere (22). For average values of the entire brain, the cerebellum and the posterior half of the pons were removed, the hemispheres were ground in a Waring blender and about 800 mgm. of the resulting mash were used for analysis.

Lumbar fluids from live persons and cisternal fluid from cadavers (4 cc. each) were worked up in the same way as the brain filtrates (22) Also protein-free brain dialyzates, redissolved dry evaporation residues, adsorbates and eluates of various types (see below) were analyzed in the same fashion, going through both adsorptions by $Al(OH)_3$ at pH 4.0 and 8.5. The acid $Al(OH)_3$ adsorption process was omitted, however, when aqueous solutions of epinephrine or arterenol (norepinephrine) were analyzed for comparison.

The colorimetric encephalin readings are expressed in gamma equivalents, each gamma equivalent corresponding to the color effect of one gamma of epinephrine.

Isolation and purification of encephalin from brain tissue. This was done essentially by dialysis adsorption and elution. At first the dialyses were carried out according to the method described by O. Loewi (27) and by Cannon and Lissák (28). Brain tissue was ground with sand; distilled water (3 cc. per gram of brain) was added; dialysis followed for four hours in a shaking apparatus in special dialysis cups through a cellophane membrane against equal amounts of distilled water. The dialyzate was then evaporated in a desiccator at room temperature. The dry residue was redissolved in distilled water (5 cc. for 10 gram of fresh brain). With this procedure 46–51 per cent of encephalin was recovered from the brain.

To obtain larger quantities the fresh brain mash was placed in cellophane tubes which were tied at the ends. These 'sausages' were immersed in a volume of n/100 HCl, amounting to three times the weight of the brain mash and, with occasional stirring, they were kept in the refrigerator for 24 hours. Then they were transferred into n/100 HCl, using half the amount of the first volume.

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[2] This material was originally believed to consist of 'adreno-cortical' compounds, a conception which was later abandoned (38).

After another 24 hours in the refrigerator, the dialyzates were pooled, filtered, placed in bottles with rubber caps (250 cc. in each), frozen and evaporated to dryness. This latter process, which was carried out by the Vermont Red Cross Blood Bank, required about three days.

The dry residue which is rather hygroscopic was kept in the refrigerator in tightly closed flasks. Weighed aliquots were dissolved in water for colorimetric assay and pharmacological testing, this procedure yielding 66–70 per cent of the encephalin contained in the fresh brain.

Since the dialyzate residue proved to contain disturbing by-products, attempts were undertaken to obtain encephalin in greater purity. Various adsorption and subsequent elution procedures were tried with the dry residue of the dialyzate after it had been redissolved in water. Successive adsorptions by $Al(OH)_3$ at pH 4.0 and 8.5 carried all the encephalin into the alkaline adsorbate, but efforts to set it free again through acidification with HCl yielded only 20–25 per cent.

Adsorptions to nonsoluble adsorbents (kaolin, fuller's earth, carbon) at pH 4.0 and 8.5, and subsequent elution from the adsorbates with HCl eliminated most of the disturbing impurities, but the yields of encephalin too were poor (table 1). Alcohol and ether extracts made from brain mash were likewise unsatisfactory, giving very low yields (1–11 per cent) and eliciting disturbing and sometimes fatal side-effects (deep fall of blood pressure, cardiac standstill).

The following procedure permitted recovery of up to 80 per cent of the encephalin. One gram of the dry residue of the acid dialyzate (see above) is dissolved in 8 cc. of water plus 4 cc. of $Al(OH)_3$, prepared according to Shaw (23), the pH is adjusted to 4.0 with 1.5 cc. n/1 HCl and the suspension is centrifuged at 3000 r.p.m. for about five minutes. To the decanted supernatant fluid one gram of fuller's earth (heated at 500° for 12 hours) is added. The suspension is stirred and centrifuged for about 10 minutes. To the decanted supernatant fluid a 4 per cent NaOH solution (about 1.4 cc.) is added to bring the pH to 8.5, and one gram of fuller's earth. After centrifuging for about 10 minutes, one gram Norit (decolorizing carbon) is added to the decanted supernatant fluid and the suspension is filtered. For storage purposes the filtrate is brought to pH 4.0 by adding n/1 HCl (about 0.6 cc.). The encephalin concentration of the filtrates thus obtained ranges between 1 and 3 gamma equ. per cc.

The main disadvantage of this procedure is the fact that the final solution contains potassium salts, amounting to 3–10 mgm. of potassium per gamma equivalent of encephalin. Attempts to remove the potassium by treatment with tartaric acid or chloroplatinic acid were unsuccessful insofar as also most of the encephalin was removed or destroyed.

Finally, the following procedure was adopted which yielded not more than 25 to 30 per cent of the encephalin but with which only 0.3 to 0.5 mgm. of potassium per gamma equivalent of encephalin remained in the ultimate eluate. The first steps are the same as outlined in the preceding paragraph. To the fluid decanted after the first centrifuging, another 4 cc. of $Al(OH)_3$ are added and about 2 cc. of 4 per cent NaOH, to bring the pH to 8.5. After centrifuging for 5 minutes the supernatant fluid is discarded, 5 cc. of water are added to the sediment, the sediment is thoroughly stirred up with a glass rod and the suspension is again centrifuged for 5 minutes. After discarding the supernatant fluid, 2 cc. of 3 per cent NaH_2PO_4 are added to the sediment and the latter is again stirred up. Eight drops of n/1 HCl are added to bring the pH to 5.0. After 15 minutes the suspension is centrifuged and the supernatant fluid is decanted and placed in a closed flask for cold storage. For injection purposes it can be neutralized with a few drops of saturated sodium bicarbonate solution. (For colorimetric assay (22) 0.25 cc. of the encephalin solution plus 2.2 cc. of water are placed in a test tube and worked up like any specimen having reached the last phase before addition of 0.35 cc. of 4 per cent NaOH. The colorimeter reading must be multiplied with 8 to obtain the concentration of encephalin per cc.)

Pharmacological tests. The effect of encephalin on the blood pressure was tested on atropinized (1 mgm/kgm.) cats under nembutal anesthesia (35–50 mgm/kgm.) and artificial respiration. The completeness of atropinization was usually checked by the failure of the blood pressure to fall after injection of acetylcholine (0.01 mgm/kgm.). The adrenal glands were tied off, the blood pressure was recorded with a mercury manometer from the carotid or femoral artery and intravenous injections were given into the femoral vein.

The dosage of encephalin was calculated from the results of colorimetric determinations carried out in the respective solutions. It was expressed in gamma equivalents and compared with the effects of equal amounts of epinephrine and arterenol (nor-epinephrine, probably identical with sympathin, (29, 30)) under a variety of conditions. The following drugs were used: adrenalin hydrochloride (1:1000), Parke, Davis; dl-arterenol (nor-epinephrine), which was kindly supplied by Dr. M. L. Tainter of the Sterling-Winthrop Research Institute; l-Corbasil (dihydroxy-nor-ephedrine) for which we are indebted to the Farbwerke Höchst, Frankfurt a.M.; tyramine (Burroughs, Wellcome & Co.); atropine sulphate (Parke, Davis); acetylcholine bromide (Eastman Kodak); gynergen (Sandoz); cocaine and dibenamine hydrochloride for which we want to thank Dr. W. Gump of Givaudan-Delawanna Chemical Laboratories.

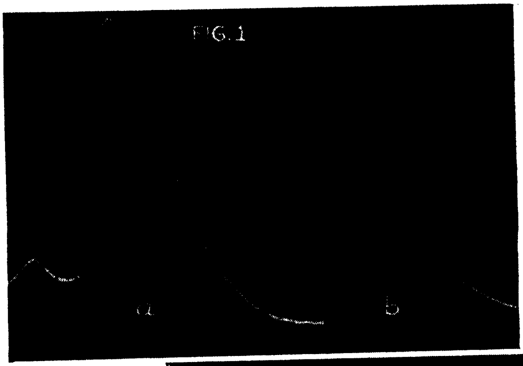
RESULTS

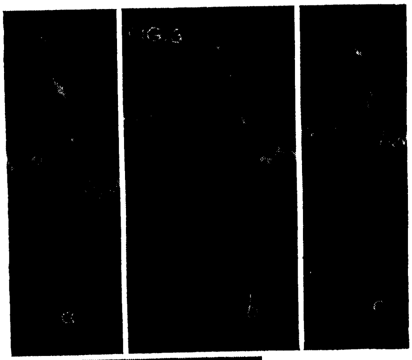
(1) *Identity of colorimetrically and biologically tested material.* Since the colorimetric and the pharmacological results of this study were largely correlated, it seems advisable to give a brief introductory account of those findings which definitely suggest the identity of the colorimetrically determined chromogenic and the biologically active material under consideration (encephalin).

(*a*) The quantitative colorimetric and vasopressor effects of equal amounts of brain dialyzate (crude or purified) were practically identical as long as fresh material was used (see section 2, fig. 1). Similar conditions prevailed regarding the effect on the rabbit's intestine and cat's uterus (sections 9, 10).

(*b*) The recoveries and losses of chromogenic and vasopressor efficiency of encephalin solutions (as tested against epinephrine standards), which occurred during various adsorption procedures and elutions ($Al(OH)_3$, fullers earth, kaolin), showed a close parallelism (fig. 2). Blood pressure effects could be approximately predicted from the calculated encephalin concentrations. A short fall of the blood pressure, preceding the characteristic rise which occurred with the injection of encephalin, adsorbed by and again eluted from $Al(OH)_3$ (fig. 2), was possibly due to contamination with traces of aluminum.

(*c*) The degree of resistance to destruction through boiling with alkali and through iodine was approximately analogous regarding the chromogenic and vasopressor properties of encephalin (see sections 13, 14).





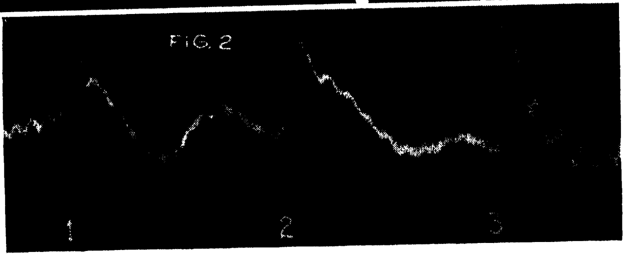


FIG. 1. CAT, atropinized, adrenals tied. *a*) brain dialyzate (8 gamma equ. encephalin); *b*) epinephrine (8 gamma).

FIG. 2. CAT, atropinized, adrenals tied. 1) epinephrine (1.5 gamma); 2) cow brain dialyzate (1.5 gamma equ. encephalin, eluted from alkaline adsorbate to $Al(OH)_3$ through treatment with HCl); 3) cow brain dialyzate (1.5 gamma equ. encephalin, crude).

FIG. 3. CAT, atropinized, adrenals tied. *a*) arterenol (20 gamma); *b*) cow brain dialyzate (20 gamma equ. encephalin, crude); *c*) human brain dialyzate (20 gamma equ. encephalin, crude). Initial falls of blood pressure due to ventricular fibrillation induced by admixture of potassium.

(2) *Blood pressure effects.* The blood pressure response of the cat to intravenous injections of fresh encephalin was practically identical with that elicited by colorimetrically identical, known doses of epinephrine and similar to that elicited by analogous (weight for weight) doses of arterenol (fig. 1, 5a, 11). It reached levels of 200 mm. Hg. (The chromogenic efficiency of arterenol was found to be only about one third of that of epinephrine. A blood pressure effect of arterenol, twice as strong as that of epinephrine as described by von Euler (30), was seen occasionally but not regularly.)

In some instances the encephalin effect differed from the effect of epinephrine or arterenol by being somewhat weaker or stronger (fig. 3, 5a, 12), occasionally also by forming a different shape of the curve apex (fig. 11). A diminished height of the encephalin blood pressure curves was observed when old material was used. The blood pressure rises after larger doses of crude encephalin were often preceded by a deep fall of the blood pressure (fig. 3). This phenomenon is caused by the toxic effect upon the heart of potassium (contained in the encephalin solutions) which elicits ventricular fibrillation. The vasopressor action of encephalin per se is not affected by the accompanying potassium as demonstrated by the inefficiency of the injection of equal amounts of potassium with equal speed (fig. 4).

Equal amounts of encephalin, obtained from the cortex, the basal ganglia and the white matter of the brain, produced identical effects on the blood pressure.

(3) *Effect of cocaine.* Pretreatment with cocaine (10 mgm/kgm. intramuscularly), while strongly intensifying the vasopressor effects of epinephrine and arterenol in the usual fashion (29, 30), left the effect of encephalin unchanged or even weakened it (fig. 5a, b).

(4) *Effects of ergotamine and dibenamine.* Pretreatment with ergotamine tartrate (4 mgm/kgm.) or with dibenamine hydrochloride (20 mgm/kgm. intraperitoneally) inverted the blood pressure effect of epinephrine but only weakened the effects of arterenol (in confirmation of von Euler's findings, 30) and of encephalin, without inverting them (fig. 6a, b, c).

(5) *Effect of decerebration and pithing.* After decerebration and pithing the vasopressor effect of encephalin was considerably weakened, as compared with that of epinephrine, but still distinctly present.

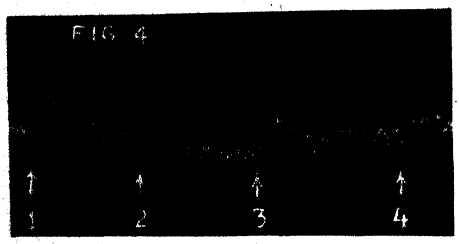
(6) *Effect on the cat's pupil.* The action of encephalin on the cat's pupil, as compared with that of epinephrine, arterenol and corbasil (dihydroxy-nor-ephedrine), was tested by injection into the carotid artery toward the eye, with the cervical sympathetic cut (fig. 7). While the effects of equipressor doses of arterenol and of corbasil were weaker than that of epinephrine, in agreement with von Euler (30), the effect of equipressor amounts of encephalin was both more intense and more prolonged than that of epinephrine.

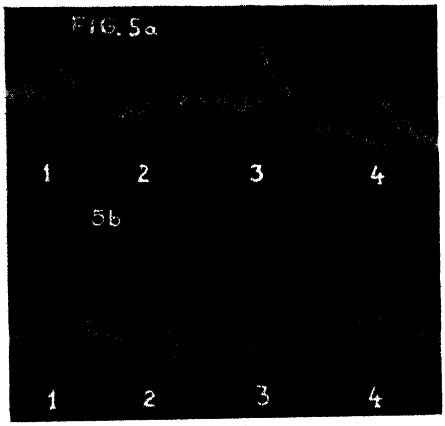
(7) *Effect on the isolated frog's heart.* Perfusion of the isolated frog's heart, according to the Straub technique, with insufficiently purified encephalin in Ringer solution at pH 7.0 caused cardiac inhibition in concentrations as low as 0.1 gamma equ./cc., probably due to the presence of excess potassium. Practically potassium-free encephalin was ineffective in frog hearts the response of which to epinephrine was also very poor.

(8) *Effect on the mammalian heart.* Perfusion of the coronary arteries of the isolated rabbit's heart (Langendorff technique, oxygenated glucose Ringer solution at 38°) with encephalin had a stimulating effect, even after the heart had come to a standstill (fig. 8). Electrocardiographic changes, elicited in the cat by the intravenous injection of potassium-free encephalin solutions, resembled those caused by epinephrine or arterenol, regarding deformation of the T waves and displacement of the S-T segment. They will be described in detail elsewhere.

(9) *Effect on the isolated rabbit's intestine.* Rabbit's duodenum, suspended in

a 25 cc. Ringer's solution at 38°, was markedly inhibited by purified encephalin in a dilution of 1:10 million, and slightly in a solution of 1:20 million. The effect was similar to that of equal amounts of epinephrine (fig. 9 a–d). Crude brain dialyzates, on the contrary, produced a stimulating effect.





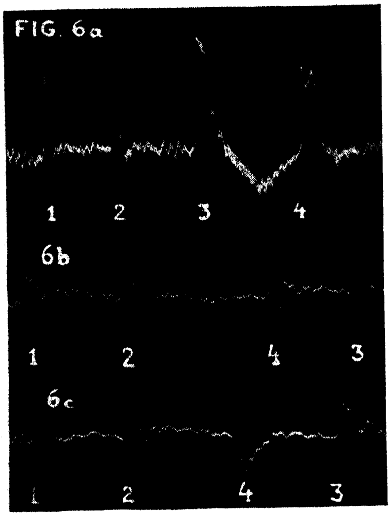


Fig. 4. Cat, atropinized, adrenals tied. Effect on blood pressure of encephalin solution containing 4 mgm. potassium per gamma equ., and of equal amounts of potassium alone (as KCl): 1) encephalin (1 gamma equ.) + 4 mgm. K (0.5 cc. injected in 1 second); 2) 4 mgm. K (0.5 cc. injected in 1 second); 3) encephalin (2 gamma equ.) + 8 mgm. K (1 cc. injected in 2 seconds); 4) 8 mgm. K (1 cc. injected in 2 seconds).

Fig. 5*a*. Cat, atropinized, adrenals tied. 1) epinephrine (1 gamma); 2) human encephalin (1 gamma equ.); 3) cow encephalin (1 gamma equ.); 4) arterenol (1 gamma). 5b. Same cat 1 hour after 34 mgm. cocaine i.m. Same sequence and dosages as above. Encephalin effect weakened.

Fig. 6*a*. Cat, atropinized, adrenals tied. 1) human encephalin (5 gamma equ.); 2) calf encephalin (5 gamma equ.); 3) arterenol (5 gamma); 4) epinephrine (5 gamma). 6*b*. 30 minutes after i.p. inj. of 20 mgm/kgm. dibenamine hydrochloride. Dosages as above. Effect of epinephrine inverted, of encephalin and arterenol only weakened. 6*c*. 10 minutes later. Each injection 10 gamma (or gamma equ.). Same type of reaction.

(10) *Effect on the isolated nonpregnant cat's uterus.* With a technique analogous to that used for the isolated intestine, encephalin (4 to 8 gamma equ. in a 25 cc. Ringer solution) was found to inhibit the cat's uterus to a somewhat lesser degree than equivalent doses of epinephrine (fig. 10 a–c). The effect of crude brain dialyzates was ambiguous.

(11) *Adsorbability and elutability of encephalin.* The adsorbability of encepha-

lin by $Al(OH)_3$, kaolin, fullers' earth and Norit (carbon) was, in general, very much like that of epinephrine and arterenol (table 1) except for some quantitative differences, especially a much lower degree of adsorbability by Norit at pH 8.5, which was made use of in the process of purification of encephalin. Elutability of encephalin from alkaline adsorbates through acidification with HCl was generally rather poor, more so than was the case with epinephrine and arterenol. It was more satisfactory in the presence of NaH_2PO_4.

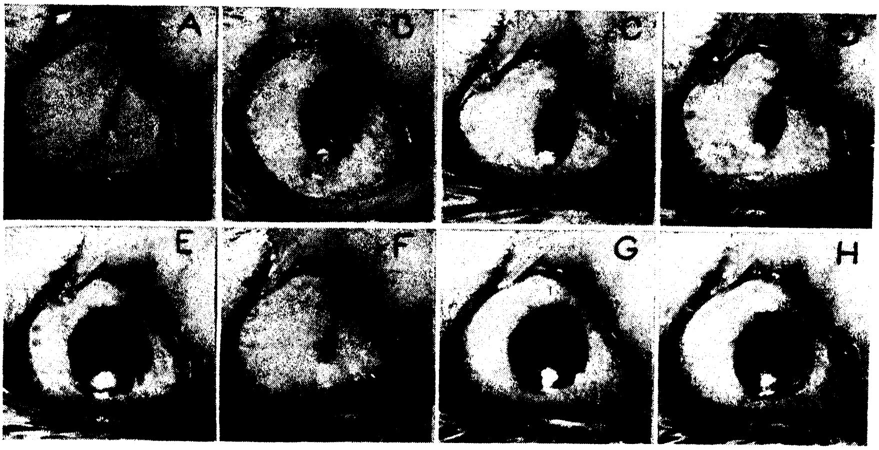


FIG. 7. CAT PUPIL (nembutal, cervical symp. cut), equipressor doses injected. *a)* control; *b)* epinephrine (5 gamma); *c)* nor-epinephrine; *d)* dihydroxy-nor-ephedrine; *e)* human encephalin; *f)* control; *g)* human encephalin; *h)* the same as *g* (1 minute later).

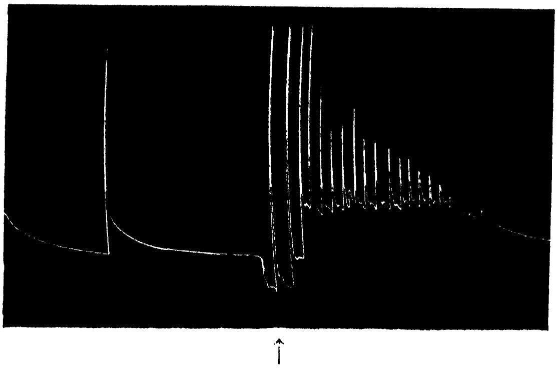
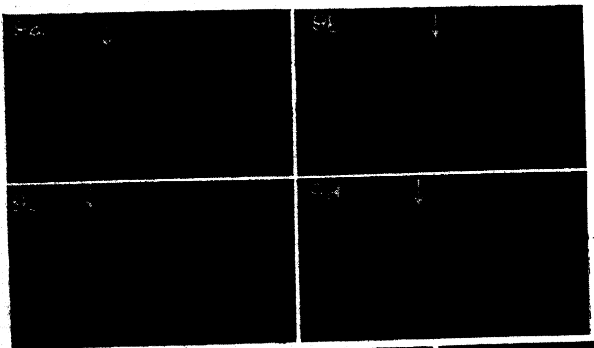


FIG. 8. ISOLATED RABBIT'S HEART perfused with glucose Ringer. After the heart had come to an almost complete standstill, at ↑ 2 gamma of encephalin (in 2 cc.) were added to the perfusion fluid.

(12) *Stability ot encephalin.* When kept in the refrigerator, the brain mash and acid dialyzates (liquid or dry) maintained their chromogenic properties for weeks, with a gradual loss of color intensity, however, amounting to about 10 per cent within one week, to about 65 per cent within 12 weeks. The diminution of vasopressor property seemed to proceed somewhat faster, so that a certain discrepancy between color effect and blood pressure effect used to appear, beginning after about 2 weeks of cold storage. The chromogenic material, present in the cerebrospinal fluid, appeared to be much more stable than that obtained



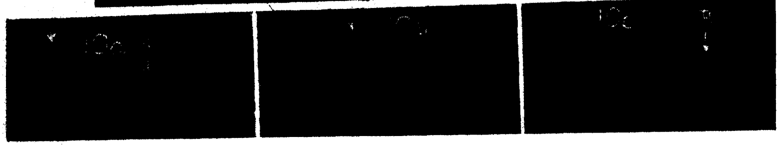


FIG. 9. ISOLATED RABBIT'S INTESTINE. 9*a*) Epinephrine (1:10 million) eluted from fullers' earth. 9*b*) cow encephalin (1:10 million) eluted from fullers' earth. 9*c*) epinephrine (1:20 million) eluted from kaolin. 9*d*) cow encephalin (1:20 million) eluted from kaolin.

FIG. 10. CAT UTERUS in 25 cc. Ringer. 10*a*) Epinephrine (4 gamma), R = fresh Ringer; 10*b*) encephalin (4 gamma equ.); 10*c*) encephalin (8 gamma equ.), R = fresh Ringer.

TABLE 1

ADSORBENT	EPINEPHRINE	ARTERENOL	ENCEPHALIN
Maximal adsorption observed at pH 4.0			
	%	%	%
$Al(OH)_3$	0	0	0
Kaolin	73	68	41
Fullers' earth	24	0	0
Norit	100	100	80
Maximal adsorption observed at pH 8.5			
$Al(OH)_3$	100	100	100
Kaolin	51	41	17
Fullers' earth	0	0	29
Norit	100	100	15
Maximal recovery[1] through acid elution with HCl from alkaline adsorbates			
$Al(OH)_3$	100	76	25
Kaolin	25	23	17
Fullers' earth	70	68	29
Norit	?	?	8

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[1] Expressed in per cent of the original total amounts used in the tests (not of the adsorbed amounts).

from brain dialyzates. It was found quantitatively unchanged after 6 months in the refrigerator and after 8 hours incubation at 37°C.

(13) *Resistance to alkali.* Exposure of epinephrine, arterenol and encephalin (0.2 gamma/cc. each) to intense alkalinization (pH 11) at room temperature (30 minutes) did not alter their color effects significantly. When boiled for 15 minutes epinephrine and arterenol lost their chromogenicity completely; encephalin lost 83 per cent of it. Slight alkalinization (pH 7.0) and boiling for 30 minutes decolorized epinephrine completely, while arterenol lost only 61 per cent and encephalin 61–77 per cent of their color effects. The diminutions of vasopressor activity roughly paralleled those of chromogenicity.

(14) *Resistance to oxidation through iodine.* Solutions of epinephrine, arterenol and encephalin (25 cc. each, 0.3–1.7 gamma/cc., pH 7.0) were mixed each with 6 drops of n/10 I_2 and left standing at room temperature for 5 hours. (In the process of colorimetric analysis the second (alkaline) $Al(OH)_3$ adsorbate had to be carefully washed several times to remove the iodine which is itself chromogenic.) Epinephrine and arterenol were completely decolorized, while the chromogenicity of encephalin solutions was somewhat increased (iodine contamination of the adsorbate ?). Correspondingly, the vasopressor effects of epinephrine and arterenol were nearly abolished, while that of encephalin remained almost unchanged (fig. 11). The possibility that encephalin might have been protected against oxidation by iodine through the presence of other accompanying substances in the brain dialyzates was made improbable by the result of mixing epinephrine with a dialyzate and subsequent addition of iodine; epinephrine was again completely destroyed.

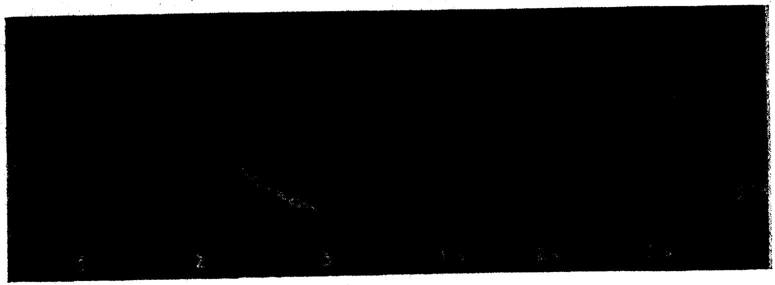
(15) *Resistance to ultraviolet irradiation.* Solutions of epinephrine, arterenol and encephalin (20 cc. each, 1 gamma/cc., pH 7.0) were exposed to a therapeutic ultraviolet lamp at a distance of 20 cm. in open Petri dishes, which were cooled by being placed on a wire netting in a water bath with running tap water. Control solutions were also kept in open Petri dishes at the same temperature. Epinephrine and arterenol lost 89 and 90 per cent, respectively, of their chromogenicity within one hour, 100 per cent within 2 hours. The color effect of encephalin on the other hand, remained unchanged. The vasopressor effect of arterenol and epinephrine was completely abolished (the latter in agreement with other observers (34, 35, 36)), that of encephalin was only weakened (fig. 12).

(16) *Color and fluorescence reactions.* Encephalin reduces arsenomolybdic acid, giving rise to a blue color. It has this effect in common with a number of substances, but adsorption by $Al(OH)_3$ at pH 4.0 eliminates some of these, e.g. glutathione, while subsequent adsorption by $Al(OH)_3$ at pH 8.5 separates encephalin, epinephrine, sympathin and related compounds with a benzene ring and two or more free hydroxyl groups in orthoposition from chromogenic contaminants which are not adsorbable at that pH. This type of combined chromogenic and adsorption specificity (see table 1) constitutes the principle upon which the colorimetric procedures for the determination of epinephrine (23, 37) and related compounds (38), including encephalin, are based.

The d.s.r. (denominator of the specific ratio (22, 38)) of encephalin was near

1.0 in almost all of 87 determinations (av. 1.02). This distinguishes it from the d.s.r.'s of epinephrine and arterenol which are nearer 2.0.

Encephalin differed from epinephrine more fundamentally regarding other color reactions. Addition of one volume of 4 per cent acetic acid and 3 volumes of 4 per cent KIO_3 (39) to one volume of epinephrine solution (100 gamma/cc.) developed a purplish red color while a similar amount of encephalin turned yellow. $FeCl_3$, added to epinephrine, produced a green color which gradually changed into orange. No discoloration took place with encephalin. The fluorescence reaction of Gaddum & Schild (52), which gives a strong green fluorescence in alkali-treated epinephrine solutions and a very weak one in alkali-treated arterenol solutions (30), was not present in brain dialyzates. They displayed at first an intense green fluorescence (unspecific ?) which disappeared after alkalinization.



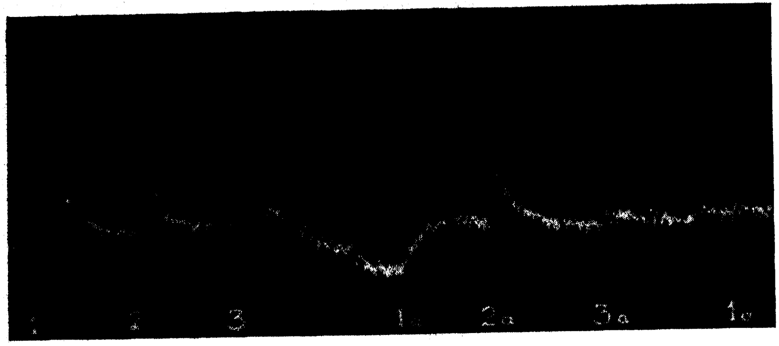


Fig. 11. (*upper*) Cat, atropinized, adrenals tied, 0.1 mgm/kgm. gynergen s.c. 1) epinephrine (2.5 gamma); 2) arterenol (2.5 gamma); 3) human encephalin (2.5 gamma equ.); 1a) epinephrine (2.5) iodine-treated; 2a) arterenol (2.5) iodine-treated; 3a) human brain (2.5) iodine-treated.

Fig. 12 (*lower*) Cat, atropinized, adrenals tied. 1) Epinephrine (5 gamma); 2) encephalin, old (5 gamma equ.); 3) arterenol (5 gamma); 1a) epinephrine (5 gamma) exposed to ultraviolet); 2a) encephalin (5 gamma equ.) exposed to ultraviolet; 3a) arterenol (5 gamma) exposed to ultraviolet.

(17) *Comparison with tyramine.* Tyramine, which, in contrast to encephalin, is not chromogenic when brought into contact with arsenomolybdic acid, was injected intravenously into atropinized cats in a dosage several hundred times larger (0.5 mgm.) than the usually effective encephalin doses. The ensuing ele-

vation of the blood pressure was much more prolonged than the customary effect of encephalin, epinephrine or arterenol; it was much more weakened by cocaine (in agreement with Tainter and Chang (33)) than the effect of encephalin, and pretreatment of tyramine with iodine inverted its effect on the blood pressure, in contrast to the iodine resistance of encephalin (fig. 11).

(18) *Distribution of encephalin in the brain and other parts of the central nervous system.* Chromogenic material of the type of encephalin was found in relatively large amounts in all parts of the central nervous system, including the choroid plexus and the cisternal and lumbar cerebrospinal fluids, but only dialyzates of the hemispheres were tested for biological effects.

The quantitative distribution of encephalin in the brains of 'normal' and diseased humans and of different animal species under various experimental conditions will be discussed elsewhere.

DISCUSSION

The chemical structure of encephalin is unknown. However, the fact that its chromogenic reaction with arsenomolybdic acid, its adsorbability by various adsorbents (table 1) and its pharmacological effects (fig. 1, 5a, 6a, 7–10) resemble those of epinephrine and of arterenol (which is supposed to be identical with sympathin, 30, 42), makes it probable that it is also chemically closely related to these sympathomimetic amines (table 2). That it is not identical with either of them, however, appears evidenced by the following differences: no intensification of the blood pressure effect through cocaine (fig. 5b); no destruction through ultraviolet light (fig. 12); no destruction through iodine (fig. 11); no epinephine-like color reactions with $FeCl_3$ and KIO_3; no epinephrine-like fluorescence reaction. Regarding inhibition by dibenamine (fig. 6b, c) and resistance to alkali, encephalin corresponds more closely with arterenol than with epinephrine.

The characteristic resistance of encephalin to oxidation by exposure to iodine or to ultraviolet light might be possibly explained by a compound formation of encephalin with lipids, as brain lipids are known to inhibit the oxidation of epinephrine (43), but there are also other sympathomimetic amines (ephedrine, sympatol, isopropylsympatol) which are more or less ultraviolet-resistant by themselves (44).

The nonidentity of encephalin with tyramine was proven by the demonstration of several fundamental discrepancies.

Basic differences between encephalin and pitressin are the following: pitressin is nonchromogenic with arsenomolybdic acid; its blood pressure effect is more prolonged and subject to tachyphylaxis; it stimulates the rabbit's intestine. Other facts which militate against an identity of encephalin with pitressin are the presence of relatively high concentrations of encephalin in the brain cortex and the relatively low concentration of chromogenic material (encephalin ?) in the pituitary gland.

On the other hand, there are some indications that encephalin may be identical with Page's (20) 'C.E.A.' (central excitatory agent) which he found in the cerebrospinal fluid, also after hypophysectomy. It had the following peculiarities in common with encephalin: vasopressor effect unaltered by cocaine and by atropine, weakened by ergotamine. It differed from encephalin by being weakened through repeated injection and by abolition of its vasopressor effect through

TABLE 2

The data concerning epinephrine and arterenol were largely compiled from the literature (28, 29, 30, 45, 48, 53, 76) but many were also based on original observations which were made in the course of this work and which were in good agreement with those given by other workers.

TYPE OF TEST	EPINEPHRINE	ARTERENOL	ENCEPHALIN
Blood pressure (cat)	Elevation (++)	Elevation (+++)	Elevation (++)
Effect of cocaine on bl. pr. reaction	Enhanced	Enhanced	Unchanged or weakened
Effect of ergotamine on bl. pr. reaction	Inverted	Weakened	Weakened
Effect of dibenamine on bl. pr. reaction	Inverted	Weakened	Weakened
Effect of boiling with alkali on bl. pr. reaction	Abolished	Weakened	Weakened
Effect of iodine on bl. pr. reaction	Almost abolished	Almost abolished	Unchanged
Effect of ultraviolet irradiation on bl. pr. reaction	Abolished	Abolished	Sl. weakened
Effect of pithing on bl. pr. reaction	Unchanged	?	Weakened
Isolated mammalian heart	Stimulated	?	Stimulated
Electrocardiogram (cat)	Altered T	Altered T	Altered T
Cat's pupil	Dilated (++)	Dilated (+)	Dilated (+++)
Isolated rabbit's intestine	Inhibited (++)	Inhibited (+)	Inhibited (+)
Isolated cat's uterus	Inhibited (++)	Inhibited (+)	Inhibited (+)
Arsenomolybdic acid test	Blue (+++)	Blue (+)	Blue (+++)
Above after boiling with alkali	No color	Weak blue	Weak blue
Above after treatment with iodine	No color	No color	Blue (+++)
Above after irradiation with ultraviolet light	No color	No color	Blue (+++)
KIO_3 test	Purplish	?	Yellow
$FeCl_3$ test	Green	Green	No color
Fluorescence	Weak green, with alkali strong	No fl., with alkali weak green	Strong green, with alkali no fl.
Adsorptions and elutions: see table 1			

pithing, while the effect of encephalin remained fairly constant with repeated injections and was only weakened by pithing.

Von Euler's (30) failure to obtain more than very small amounts of a sympathomimetic substance from the brain of a calf is probably due to the fact that encephalin cannot be isolated from the brain in significant quantities by alcoholic extraction, as our own experience suggested.

The presence of what seems to be encephalin in the cerebrospinal fluid can be assumed to be responsible for at least part of the vasopressor properties of this fluid which are usually attributed to secretion of pitressin from the posterior pituitary lobe into the third ventricle. The exact way in which encephalin passes from the brain tissue into the cerebrospinal fluid is not known but its relatively high concentration in the choroid plexus suggests that at least a substantial portion of it is being secreted from there into the ventricular cavities.

Certain neuro-vegetative reactions, analogous to the typical effects of encephalin which occur in animals after stimulation of the hypothalamus (elevation of blood pressure, inhibition of intestinal motility, pupillary dilatation (45, 46, 47)), have been interpreted by some investigators as being due to hypophyseal or adrenal hormonal discharges, but it appears possible that encephalin discharges from the brain via the cerebrospinal fluid into the blood circulation may play a part in the origin and prolonged maintenance of such hypothalamic phenomena. Morphological indications of a neurosecretory activity of the diencephalic area of the brain have been described by several workers (41, 49, 50, 51).

The 50 per cent lower concentration of what appears to be encephalin in the lumbar fluid, as compared with that in the cisternal fluid, seems to indicate a diffusion of encephalin from the subarachnoidal space into the blood stream. That the difference between the concentrations in the cisternal and lumbar fluid is not due to disintegration of encephalin within the cerebrospinal canal is suggested by the fact that the concentration of chromogenic material present in the cisternal fluid was found unchanged after 8 hours of incubation at 37°C.

Physiological, psychosomatic and psychochemical implications of the presence in the brain of a potent sympathomimetic amine with the characteristics of encephalin are manifold. Some of them are being studied at the present time.

SUMMARY

A sympathomimetic amine was isolated in relatively large quantities from all parts of the human brain and from animal brains.

This substance has some chromogenic and most pharmacodynamic properties in common with epinephrine and with nor-epinephrine supposedly identical with sympathin), but it also differs from both of these in some significant respects. For distinction it has been designated as 'encephalin'.

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REFERENCES

1. Osborne, W. A. and S. Vincent. J. of Physiol. **25:** 283, 1900.
2. Leimdörfer, A. Wien. klin. Wschr. **43:** 1336, 1930.
3. Major, R. H. and C. J. Weber. J. Pharm. and Exp. Ther. **37:** 367, 1929.
4. Major, R. H., J. B. Nanninga, and C. J. Weber. J. of Physiol. **76:** 487, 1932.
5. von Euler, U. S. and J. H. Gaddum. J. of Physiol. **72:** 74, 1931.
6. von Euler, U. S. Arch. exp. Path. u. Pharm. **181:** 181, 1936.
7. Abel, J. J. and E. M. K. Geiling. J. Pharm. **22:** 317, 1924.
8. Sato, G. Arch. exp. Path. u. Pharm. **131:** 45, 1928.
9. Trendelenburg, P. Klin. Wschr. II, 1679, 1928.
10. Raab, W. and E. Kerschbaum. Ztschr. f. d. ges. exp. Med. **90:** 729, 1933.
11. Jores, A. and Velde: quoted by Jores, A. Klin. Wschr. II, 1599, 1933.
12. Dixon, W. E. J. of Physiol. **57:** 129, 1923.
13. Trendelenburg, P. Klin. Wschr. I, 777, 1924; Arch. f. exp. Path. u. Pharm. **114:** 225, 1926.
14. Miura, Y. Arch. f. d. ges. Physiol. **207:** 76, 1925.
15. Deleonardi, S. Arch. f. exp. Path. u. Pharm. **180:** 135, 1936.
16. Cushing, H. and E. Goetsch. This Journal **27:** 1, 61, 1910.
17. Zondek, B. and H. Krohn. Klin. Wschr. I, 405, **849:** II, 1293, 1932.
18. Oehme, C. and M. Oehme. Dtsch. Arch. f. klin. Med. **127:** 261, 1918.
19. Hülse, W. Arch. f. exp. Path. u. Pharm. **146:** 282, 1929.
20. Page, I. H. This Journal **120:** 392, 1937.
21. Clark, G. and S. C. Wang. This Journal **127:** 597, 1939.
22. Raab, W. Exp. Med. and Surg. **1:** 188, 1943.
23. Shaw, F. H. Biochem. J. **32:** 19, 1938.
24. Raab, W. Endocrinology **29:** 126, 1941.
25. Cow, D. J. of Physiol. **49:** 367, 1915.
26. Hoyle, C. Quart. J. Med. N. S. **2:** 549, 1933.
27. Loewi, O. Arch. f. d. ges. Physiol. **189:** 239, 1921; **237:** 504, 1936.
28. Cannon, W. B. and K. Lissák. This Journal **125:** 765, 1939.
29. von Euler, U. S. Acta physiol. Scandinav. **11:** 168, 1946.
30. von Euler, U. S. Acta physiol. Scandinav. **12:** 73, 1946.
31. Winkler, A. W., H. E. Hoff and P. K. Smith. This Journal **124:** 478, 1938.
32. Chamberlain, F. L., J. Scudder and R. L. Zwemer. Am. Heart J. **18:** 458, 1939.
33. Tainter, M. L. and D. K. Chang. J. Pharm. and Exp. Ther. **30:** 193, 1927.
34. Verda, D. J., Kneer, L. and Burge, W. E. J. Pharm. and Exp. Ther. **42:** 383, 1931.
35. Ewing, P. L. J. Pharm. and Exp. Ther. **48:** 273, 1933.
36. Konzett, H. and W. Weis. Arch. f. exp. Path. u. Pharm. **193:** 440, 1939.
37. Whitehorn, J. C. J. Biol. Chem. **108:** 633, 1935.
38. Raab, W. Biochem. J. **37:** 470, 1943.
39. Richter, D. and H. Blaschko. J. Chem. Soc. 601, 1937.
40. Bohn, H. Vhdlgn. d. dtsch. Ges. f. Kreislaufforschg. **6:** 218, 1933.
41. Scharrer, E. and B. Scharrer. Physiol. Reviews **25:** 171, 1945.
42. Gaddum, J. H. and L. G. Goodwin. J. of Physiol. **105:** 357, 1947.
43. Lissák, K. Arb. d. II. (med. naturwissensch.) Abtlg. d. wissensch. Tisza István Ges. in Debreczen (Hungary), Special volume, p. 1, 1942.
44. Konzett, H. Arch. f. exp. Path. u. Pharm. **198:** 361, 1941.
45. Karplus, J. P. Dtsch. Ztschr. f. Nervenheilk. **106:** 213, 1928.

46. Kabat, H., B. J. Anson, H. W. Mangoun and S. W. Ranson. This Journal **112:** 214, 1935.
47. Ranson, S. W., H. Kabat, and H. W. Mangoun. Arch. Neurol and Psych. **33:** 467, 1935.
48. Barger, G. and H. H. Dale. J. of Physiol. **41:** 19, 1910.
49. Poppi, U. Riv. Neurol. **8:** 354, 1935.
50. Gaupp, R. and E. Scharrer. Ztschr. f. Neurol. **53:** 327, 1935.
51. Roussy, G. and M. Mosinger. Cpt. rend. soc. biol. **119:** 929, 1935.
52. Gaddum, J. H. and H. Schild. J. Physiol. **80:** 9P, 1934.
53. Fröhlich, A. and O. Löwi. Arch. f. exp. Path. u. Pharm. **62:** 159, 1910.

CORONARY SINUS CATHETERIZATION FOR STUDYING CORONARY BLOOD FLOW AND MYOCARDIAL METABOLISM

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The studies reported in this and the following paper are the result of a combination of two new experimental procedures, each of which was developed and proven practical independently of the other. These are: *a*) a method for collecting coronary venous blood samples by catheterizing the coronary sinus of the dog without opening the chest (1), and *b*) application of the nitrous oxide method, developed by Kety and Schmidt for measuring cerebral blood flow (2) to the dog's coronary circulation (3, 4).

Although incidental catheterization of the coronary venous system in man has been reported by Sosman and Dexter (5), and by Bing and coworkers (6), the procedure apparently had not been employed deliberately, either in animals or man, at the time these experiments were undertaken. The desirability of data obtainable from analysis of coronary venous blood was impressed on one of us (W. T. G.) relative to the study of a case of beri-beri heart disease in man (7). Any biochemical basis for the cardiac weakness here is presumably an interference with pyruvic acid metabolism from thiamine deficiency (8). The level and exchange of pyruvic acid and related metabolites, such as glucose, lactic acid, oxygen and carbon dioxide in coronary venous and arterial blood should be of importance in further study of this condition. Similar data may well offer valuable additional information concerning myocardial failure from other causes. When an opportunity was subsequently presented for exploring the practicability of intentional catheterization of the coronary sinus of the dog, in conjunction with a study of the effects of toxic agents, it was therefore accepted.

Harrison and coworkers (9) were able to cannulate the coronary sinus of intact morphinized dogs using a modified Morawitz brass cannula (10), with a balloon inflated to divert the entire coronary sinus outflow for direct measurement. Coronary sinus outflow, however, is an inconstant fraction of the total coronary blood flow (11, 12). Furthermore, a less traumatic technic was needed, particularly for similar current studies in man (6), preferably a method involving no significant resistance to the coronary venous return. The small flexible Forssman intravenous catheter (13), as modified by Cournand (14, 15), was therefore used in the present studies.

The procedure described in this report was then developed (1), when reports of recent applications of the nitrous oxide method to the measurement of cerebral blood flow in various diseases (16, 17) suggested that the same method might be combined with the present coronary sinus catheterization technic to permit measurement of coronary blood flow, and hence the oxygen consumption and cardiac efficiency, in the intact dog, preliminary to trial of similar procedures in man (6). The nitrous oxide method avoids the major objection to previous measurements through the coronary sinus which depended upon the absolute volume of sinus outflow, for only *representative samples* of arterial and venous blood are required, such as would be obtained through a small nonoccluding catheter. The coronary blood flow would then be calculated as flow per 100 grams of myocardium drained by the coronary sinus, on the basis of integrated concentrations of nitrous oxide in coronary venous and arterial blood over a 10-minute period of gas inhalation, by an application of the Fick principle (2). The practicability of measuring coronary blood flow by the N_2O method had already been established in the dog by direct cannulation (3), and the indirect method calibrated against simultaneous direct measurements with the bubble flowmeter (4), but no measurements of the coronary blood flow had ever been made in the intact state which the present catheter technique made possible. The special experience and equipment of the two groups were therefore combined in a joint project, and the results in regard to coronary flow and related findings are presented in the second paper of this series (18). This paper deals with the technic and possible hazards of coronary sinus catheterization in the dog and with observations on the biochemical characteristics of coronary venous blood. A third phase of the same joint project, the application of both procedures to similar studies in man, is in progress, in cooperation with Bing and his collaborators at Johns Hopkins Hospital.

Anesthesia and general observations. Pentobarbital sodium, 25 mgm/kgm., was injected slowly intravenously before the procedure, and the dog was maintained in a stage of light anesthesia, with an active lid reflex, a fairly constant pulse and blood pressure and a respiratory rate and pulmonary ventilation adequate to insure a normal arterial oxygen saturation. Arterial hematocrit, hemoglobin content and oxygen saturation (19) were measured frequently throughout each experiment. Although comparable catheterization procedures have been done in unanesthetized animals (20, 21), anesthesia was used in this study chiefly for convenience and to provide a reasonably reproducible and steady status of the circulation (21, 22).

Coronary sinus catheterization. The dog, lightly anesthetized, was placed on the operating-fluoroscopy table and the neck shaved, scrubbed with sponges soaked in alcoholic zephiran solution and draped. Clean but not aseptic technique was followed throughout, with all instruments, catheters and gloves soaked in 1:600 zephiran.

A branch of the external jugular vein was exposed as high as possible in the neck and incised. A curved-tip intravenous catheter (sizes 7–9 F) was then inserted a short distance, and a slow, constant saline drip, containing 20 mgm. of heparin per liter, was connected to prevent clotting of blood in the catheter.

With the dog in the right anterior oblique position, the catheter was passed into the right auricle under fluoroscopic control. Once inside the superior vena cava, the curved tip was pointed anteromedially to avoid catching it on the ostium of the azygos vein or on the thickened ridge of muscle extending across the posterior auricular wall immediately above the foramen ovale. When the tip reached the inferior portion of the right auricle, near the tricuspid valve, it could be turned posteriorly and easily passed into the inferior vena cava (fig. 1). At this point, with the dog still in the right anterior oblique position, a triangular area of lung with the following boundaries was visible: *a*) the anteromedial border of the inferior vena cava, *b*) the posteroinferior cardiac border, and *c*) the diaphragm. The coronary sinus ostium lies just anteromedial to the superior corner of this triangle, which marks the junction of inferior vena cava and right auricle and which lies posteroinferior to the tricuspid valve. The catheter was now slowly withdrawn from the inferior cava to a point just inside the right auricle. If the tip was then turned and shifted slightly anteromedially, it pointed directly toward the coronary sinus ostium.

After one or two gentle thrusts, the catheter usually rounded a sharp initial bend (fig. 1A) and entered the sinus. The tip often passed further into the sinus, superiorly and to the left along the posterior auriculo-ventricular groove (fig. 1B) and sometimes beyond one or more delicate valves into the great cardiac vein (fig. 1C). Occasionally the ostium of the middle cardiac vein (fig. 5), located just inside the ostium of the coronary sinus (fig. 4), or more rarely, a posterior vein of the left ventricle (fig. 6), was accidentally catheterized. These veins pass along the posteroinferior septal surface toward the apex, anastomosing with each other and with the anterior descending branch of the great cardiac vein, as shown by diodrast injection in figure 6. By preference, however, only the proximal coronary sinus itself now is catheterized (fig. 1A and 1B), as discussed below, because of the dangers and technical invalidity of coronary venous obstruction.

The advantage of the right anterior oblique position for visualization of the path of the catheter into the coronary sinus is illustrated in figure 2B. In this view, a sharp bend was seen in the course of the catheter as it entered the sinus. The curve appeared foreshortened and could be seen in full length only in a more posteroanterior position (fig. 2C and 2D). In the latter view, however, the pathway was almost superimposable upon the path of a catheter entering the right ventricle and pulmonary artery, and it became extremely difficult to tell exactly where the catheter was. The chief difference between the two pathways was the more posterior orientation of the coronary sinus, which became distinct as the animal was rotated to the oblique position. In this view, furthermore, the closest and most prominent landmark to the position of the coronary sinus ostium, namely the lung triangle and inferior vena cava, were well visualized. If the dog was turned too far toward the right lateral position (fig. 2A), the pathway into the coronary sinus became difficult to visualize because of a loss of three-dimensional perspective.

Coronary venograms. Diodrast (3,5 diiodo-4-pyridone-N-acetic acid and diethanolamine) has been injected forcibly through the catheter against the stream of coronary sinus flow, and, as shown in figure 4A and 6, a retrograde coronary venogram obtained. If the catheter obstructed the vein, as in figure 5 and 6, diodrast could be easily made to fill most of the veins draining into the coronary sinus, due to the multiple veno-venous anastomoses. If the catheter lay freely in the coronary sinus, the diodrast was carried back along the shaft

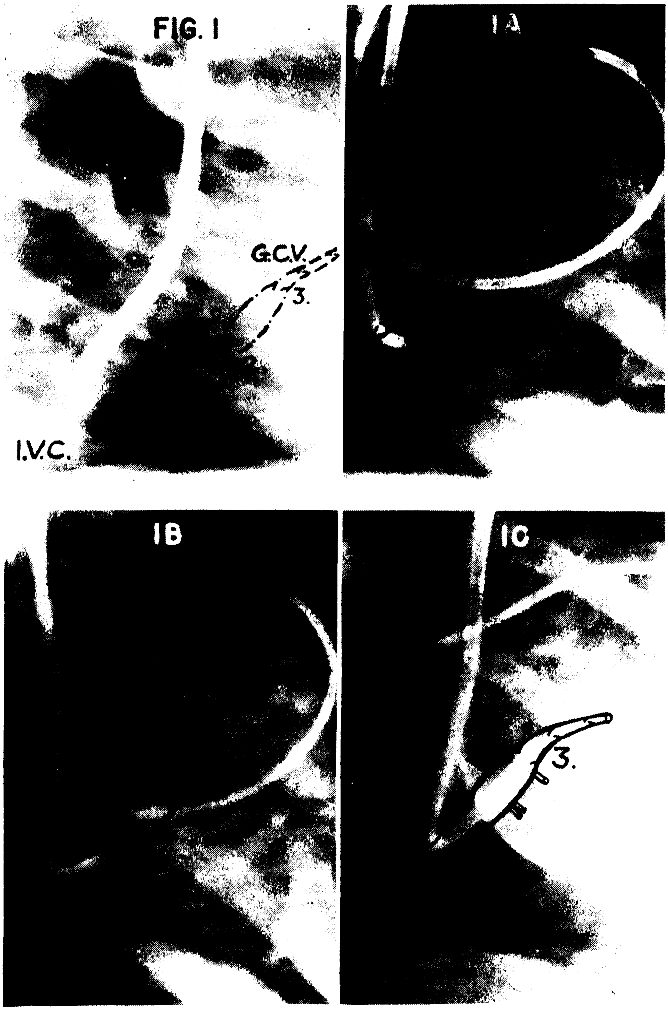
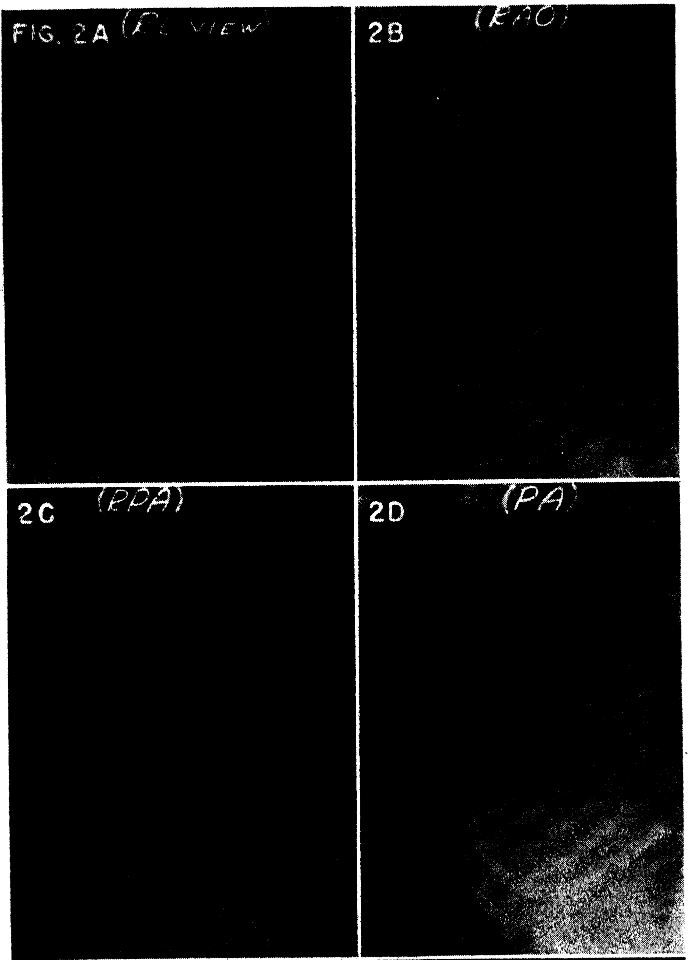


Fig. 1. Catheter inserted into the inferior vena cava (I.V.C.), illustrating the lung triangle (L.T.) and its relationship to the coronary sinus (C.S.) and great cardiac vein (G.C.V.) in the right anterior oblique view.

Fig. 1A. Catheter inserted one to 1.5 cm. into coronary sinus. *Position 1.*

Fig. 1B. Catheter inserted two to 2.5 cm., just beyond the sharp bend in the sinus. *Position 2.*

Fig. 1C. Catheter inserted into great cardiac vein, more than three cm. beyond the sinus ostium. *Position 3.*



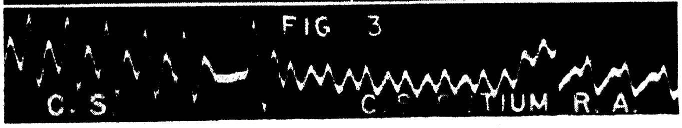


Fig. 2A–D. Comparison of right lateral (RL), right anterior oblique (RAO), right posteroanterior (RPA) and posteroanterior (PA) in the same dog. The large catheter is inserted 4 to 5 cm. into the coronary sinus, the small catheter into the pulmonary artery. The advantage of the RAO view for distinctive visualization of the catheter in the coronary sinus is illustrated.

Fig. 3. Pulse pressure curves, slightly damped, during withdrawal of the catheter from the coronary sinus, recorded through a Statham strain gauge on a Sanborn cardiette amplifier. Scale: one cm. excursion = 6 mm. Hg pressure. Systolic pulse pressures: coronary sinus (position 3), 11 mm. Hg; coronary sinus ostium (position 1), 5 mm. Hg; right auricle, 5 mm. Hg. Autopsy in this case showed that the catheter inserted to position 3, where the elevated pressure was noted as illustrated, probably caused a significant degree of coronary venous obstruction.

of the catheter in a visible stream with the normal coronary sinus flow until rapidly dispersed into the auricle at the coronary sinus ostium. The use of diodrast has helped to establish the orientation of the coronary sinus and coronary venous system as seen fluoroscopically, as well as the in vivo relationship of the coronary sinus ostium to the tricuspid valve (fig. 4A). Forcible injection is hazardous, however, since the dye may be forced into the tissues and cause local myocardial necrosis (fig. 5).

Design of coronary sinus catheters. The ordinary curved tip intravenous catheter presented the following drawbacks when used for catheterization of the coronary sinus:

a) Although fluid could always be freely injected through the catheter into the sinus, withdrawal of blood samples was often difficult or impossible. It is likely that the wall of the sinus, or a valve leaflet in the sinus or great cardiac vein, was drawn against the single catheter opening when suction was applied. Often blood could only be withdrawn either at the height of inspiration, when the descent of the diaphragm altered the position of the heart and of the catheter tip in the sinus, or during systole when the coronary venous pressure rose. This difficulty has been more serious, both in dogs and in man, when a small catheter (#6 or #7 F) has been used.

b) A small catheter (#6 or #7 F) would be most desirable for avoiding coronary venous obstruction. Such small catheters, however, were much harder to insert into the sinus than the larger sizes, because they tend to buckle in the auricle without passing around the sharp bend in the sinus. The ideal catheter then should have a small flexible tip for the part actually within the sinus and a stiff, nonbuckling shaft. This would give the best control on insertion and the least obstruction and trauma to the coronary sinus. A larger, stiffer catheter may be actually less traumatic than a smaller one which is less easily controlled because of buckling (23). A stylet, inserted to within 3–4 cm. of the catheter tip, has been used in many instances with some improvement in control, but the use of a stylet creates other technical difficulties without solving the problem of obstruction of the catheter opening.

A modified catheter has largely solved these difficulties. The shaft tapers from a stiff #8F to a more flexible #6 or #7F between 4 and 6 cm. from the tip, and there are two additional side eyes 2 mm. back from the usual terminal eye. The side eyes tend to break any possible suction on a vessel wall or valve leaflet as a sample is withdrawn. The tendency to buckle is minimal with the larger shaft, while the smaller tip causes less trauma and obstruction within the coronary sinus. (These modified catheters are available on special order from the U. S. Catheter and Instrument Co., Glens Falls, N. Y.).

Care of catheters. The original stiffness of the intravenous catheter shaft is essential to its controlled manipulation and should be restored after use by drying the catheter in an oven at approximately 90°C. for one to two hours. An inlying stylet, curved at the tip as desired, helps to keep the catheter in shape while drying. The stylet is removed after the catheter cools.

Clotting in the tip, particularly in catheters with multiple openings, may be avoided by soaking the tip in heparin solution for 30 minutes before use. The hydrophilic plastic enamel of the catheter will take up a trace of the solution. Finally, just before use, the entire lumen is flushed with the same heparin solution. Clotting in the tip may also be prevented by coating it with Silicone (dri film #9987, available from General Electric Co., Schenectady, N. Y.).

Catheterization of the pulmonary artery. For sampling mixed venous blood, a #6 or #7 F, curved-tip intravenous catheter was inserted into the same vein used for the coronary sinus catheter. By clamping the opposite sides of the vein walls between the two catheters, retrograde bleeding could largely be prevented. A saline drip, containing 10 mgm. of heparin per liter, was connected, and the catheter was then inserted into the pulmonary artery by the technique of Kinney, Haynes and Dexter (24). The tip was left far enough out into the lung field to be sure of its position, but not so far out, as Dexter and coworkers have shown can be done (23), as to draw fully oxygenated blood from the pulmonary capillaries or possible precapillary arteriovenous anastomoses.

Catheterization of the femoral artery. The femoral artery was exposed low in the femoral canal, and a #6 or #7 catheter inserted through a small incision in the artery, passed up into the aorta and tied in place. The catheter was connected to a mercury manometer for pressure recordings except during sampling.

Sampling and analytical methods. Blood samples were drawn simultaneously from the femoral and pulmonary arteries and coronary sinus through manifold systems (25) and kept ice-cold until analyzed. *Blood oxygen content* was measured by the method of Roughton and Scholander (26), checked against the method of Van Slyke and Neill (27). The latter method was also used for *carbon dioxide content* (27). *Coronary blood flow* was determined by the nitrous oxide method of Kety and Schmidt (2, 25), as applied to the coronary circulation in the accompanying report (18). Glucose samples were analyzed by Nelson's method (28), using Somogyi's copper reagent (29), pyruvic acid by the method of Friedemann and Haugen (30) and lactic acid as outlined by Barker and Summerson (31).

RESULTS

The coronary sinuses of 45 dogs, weighing 15 to 40 kgm., have been catheterized 68 times by this technique. There have been three failures, two of which were in dogs under 15 kgm. The procedure has permitted repeated observations of coronary blood flow and myocardial metabolism in the same dogs as often as seven times over a four-month period, as illustrated in the protocol of *dog #7* in figure 4 and table 1. The coronary blood flow averaged 71 cc. per 100 grams of left ventricular myocardium per minute, with a myocardial oxygen consumption averaging 9.7 cc. per 100 grams per minute. In current experiments, these observations together with an estimation of cardiac work permit the calculation of the overall mechanical efficiency of the heart.

A. *Evidence of successful coronary sinus catheterization,* (in addition to fluoroscopy). 1. The dark color of coronary sinus blood samples, corresponding to a very low oxygen saturation, clearly distinguished it even on inspection from other venous blood samples. The oxygen content of coronary venous blood averaged 4.1 volumes per cent (approximately 22 per cent saturated), as compared with a mixed venous content of 13.0 and an arterial content of 17.2. The mean coronary arteriovenous oxygen difference was thus approximately three times the total systemic A-V difference, (table 2).

2. A typical pulse pressure pattern (fig. 3) was seen when the catheter was inserted well into the coronary sinus or great cardiac vein, correlated with a marked pulsation of the blood as withdrawn or pulsation of the intravenous drip. This finding was considered to indicate at least partial coronary venous obstruction, confirmed by the elevated pulse pressure of 9–20 mm. Hg compared with a right auricular pressure of 3 to 5 mm. Pulse pressures on insertion of a small catheter a similar distance, or on withdrawal of a larger catheter to position 1, just inside the coronary sinus ostium, were no more than 2 to 3 mm. higher than right auricular pressures (fig. 1A), with minimal visible evidence of pulsation in the intravenous drip.

TABLE 1. RESULTS OF REPEATED CORONARY SINUS CATHETERIZATIONS OF THE SAME NORMAL DOG

Dog #7, male, body weight 31 kgm., heart wt. 240 gram

(See protocol, fig. 4)

DATE, 1947	17 JUNE		8 JULY		29 JULY	19 AUG.		AVE.
Experiment No.	1	2	1	2	1	1	2	
Coronary Blood Flow, cc./100 gm./min	65	58	91	77	56	79	71	71
Cardiac O_2 Consumption, cc./100 Gm./min	8.7	8.2	12.4	10.5	8.3	9.8	10.1	9.7
Arterial O_2 Content, vols. %	15.4	16.3	17.0	16.6	18.5	16.5	18.2	16.9
Coronary Venous O_2, vols. %	2.0	2.2	3.3	2.9	3.5	4.1	3.7	3.1
Mean Arterial B. P., mm. Hg	135	147	142	146	142	142	115	138
Cardiac Rate Per Min	155	160	135	130	140	160	156	148

B. *Biochemical characteristics of coronary venous blood.* Analysis of coronary venous blood clearly distinguished it from mixed venous or arterial blood, (table 2). No significant difference in these values was found when the catheter was inserted deeply into the sinus or great cardiac vein instead of only one to 2 cm. inside the ostium. The consistently high coronary arteriovenous differences of oxygen, lactate and pyruvate, considering the rate of coronary blood flow, indicate an extremely high rate of myocardial utilization of these metabolites in the normal, intact, lightly anesthetized dog. The rate of carbon dioxide production is correspondingly high. This confirms previous findings of many investigators who have used open chest and heart-lung preparations, (32–34).

Glucose was removed by the heart in relatively small amounts, often not at all, and highly inconsistently as shown by the large standard error in table 2.

Because of the interest in the constancy of the lactate/pyruvate ratio the relationship has been calculated on a small series of arterial bloods ($n = 11$)

yielding a correlation coefficient of 0.88, and a mean L/P ratio of 8. No significant difference in the ratio was found in coronary venous blood.

The cardiac lactic and pyruvic acid utilization alone could account for 40 to 60 per cent of the total cardiac oxygen utilization in these experiments, assuming eventual complete oxidation of these metabolites to carbon dioxide and water.

C. *The anatomy of the coronary sinus venous system of the dog.* Twenty-eight anatomical dissections, correlated with diodrast injections in vivo, have helped to clarify the position of the catheter with relation to the coronary sinus venous system as seen fluoroscopically (figs. 4–6).

The coronary sinus lies in the posterior auriculoventricular groove and is surrounded by epicardial fat. The sinus takes origin embryologically from the junction of the great cardiac vein with the oblique vein of Marshall. The latter is the remnant of the fetal left superior vena cava and is often difficult to identify in the adult dog. We therefore measured the length of the coronary sinus to the first valve, which was usually bicuspid and well formed and which often coincided with a sharp change in the color of the wall from dark to light. This color change is caused by a marked thinning of the muscularis. In the absence of a demonstrable valve, the point of color change alone is used as a guide. With these criteria the length of the coronary sinus measures 2 to 5 cm. and is only roughly related to the size of the heart. The catheter may appear fluoroscopically to be inserted somewhat more deeply, since it follows and probably distends the outer wall of the coronary sinus as it curves around the heart. Beyond the first valve there are often one to four single or bicuspid valves in rapid succession (fig. 1). The auricular ostium of the coronary sinus, however, is not guarded by the well-defined Thebesian valve usually found in man.

TABLE 2. BIOCHEMICAL CHARACTERISTICS OF CORONARY SINUS BLOOD

	n	MIXED VENOUS	ARTERIAL	CORONARY SINUS	MEAN ARTERIOVENOUS DIFFERENCE OF SIMULTANEOUS OBSERVATIONS[1]	
					Systemic	Coronary
		mean ± σ_m	mean ± σ_m	mean ± σ_m	mean + σ_m	mean ± σ_m
Oxygen, vols. %	21	13.0 ±.50	17.2 ±.42	4.1 ±.19	+4.2 ±.39	+13.0 ±5.4
Carbon dioxide, vols. %	10	35.9 ±1.20	31.8 ±1.20	44.5 ±1.26	−4.1 ±.19	−12.6 ±.71
Lactic acid, mgm. %	20	15.8 ±2.1	15.3 ±2.0	9.0 ±1.1	−.50 ±.30	+6.5 ±.99
Pyruvic acid, mgm. %	14	1.93 ±.14	1.99 ±.17	1.07 ±.10	+.07 ±.09	+.93 ±.15
Glucose, mgm. %	16	78.0 ±3.3	79.3 ±4.1	75.3 ±3.6	+1.3 ±2.5	+4.0 ±2.6

[1] The difference between each pair of simultaneously drawn samples was obtained and the average of these differences determined.

The middle cardiac vein enters the sinus not more than 0.5 cm. from the

FIG. 4

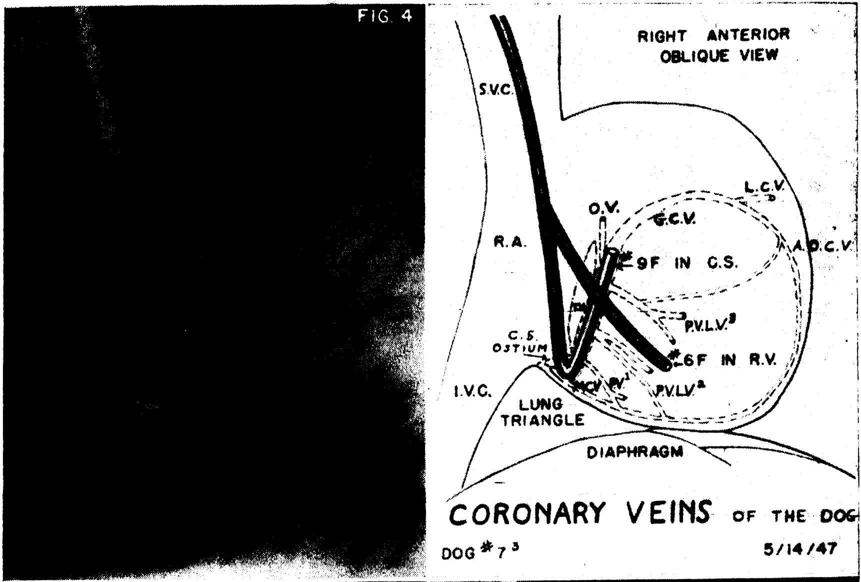


FIG. 5

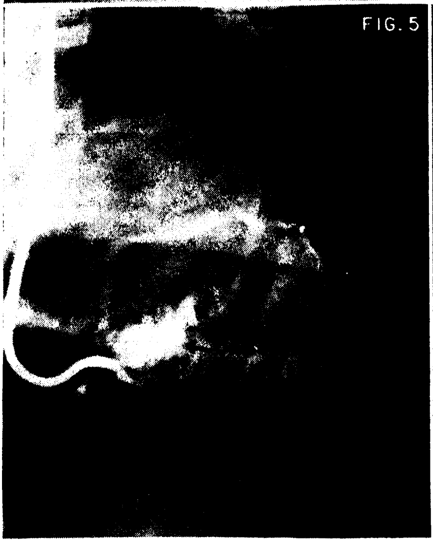


FIG. 4A R. LATERAL

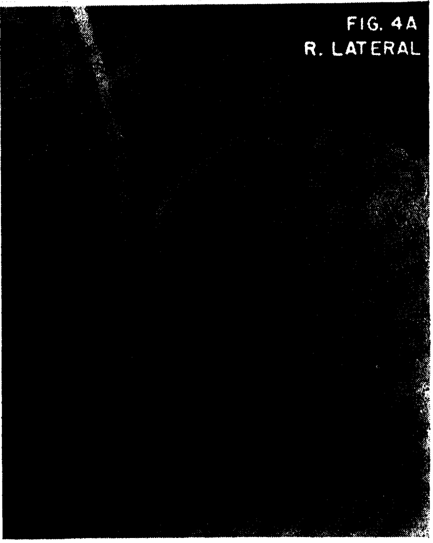


FIG. 4. THE ANATOMY OF THE CORONARY SINUS VENOUS SYSTEM in relation to a large #9 catheter, inserted three to four cm. into the coronary sinus (C.S.), with a small #6 catheter passing through the tricuspid valve (—·—·—·) into the right ventricle (R.V.). The coronary sinus venous system, as found at autopsy, is sketched as follows: middle cardiac vein, M.C.V.; posterior veins of the left ventricle, P.V.¹, P.V.L.V.², P.V.L.V.³; oblique vein of Marshall, O.V.; great cardiac vein, G.C.V.; left circumflex vein, L.C.V.; anterior descending coronary vein, A.D.C.V. The extensive veno-venous anastomoses between the major veins draining the left ventricular myocardium are illustrated.

Metabolic observations. See table 1.

Autopsy. This dog was catheterized seven times in five months. The catheter was in the sinus a total of 16 hours, and there was usually a second catheter in the pulmonary artery or right ventricle. Autopsy seven weeks after the last procedure showed a normal heart except for slight subendocardial fibrosis in the right auricle and thickening of the medial tricuspid valve leaflet.

FIG. 4A. DIODRAST INJECTION OUTLINING THE GREAT CARDIAC VEIN and its branches. Arrow points to diodrast flowing with the coronary venous return from the coronary sinus ostium through the tricuspid valve.

FIG. 5. CATHETER OBSTRUCTING THE MIDDLE CARDIAC VEIN. The shadow of diodrast in

auricle. It drains the posterior septal area and often a small rim of right ventricle and is often too small to admit a catheter tip. Two to four major posterior left ventricular veins open into the sinus between one and 4 cm. from its auricular ostium. These veins vary in size and number and often have multiple openings into the coronary sinus. There are usually abundant veno-venous anastomoses, not only among the middle and posterior veins, but also between one or more of these veins and the anterior descending vein near the apex. The veno-venous anastomoses are best demonstrated by diodrast injection in vivo, (figs. 4A–6). Beyond the last posterior vein for a distance of 1.25 to 3 cm., there are no significant venous junctions.

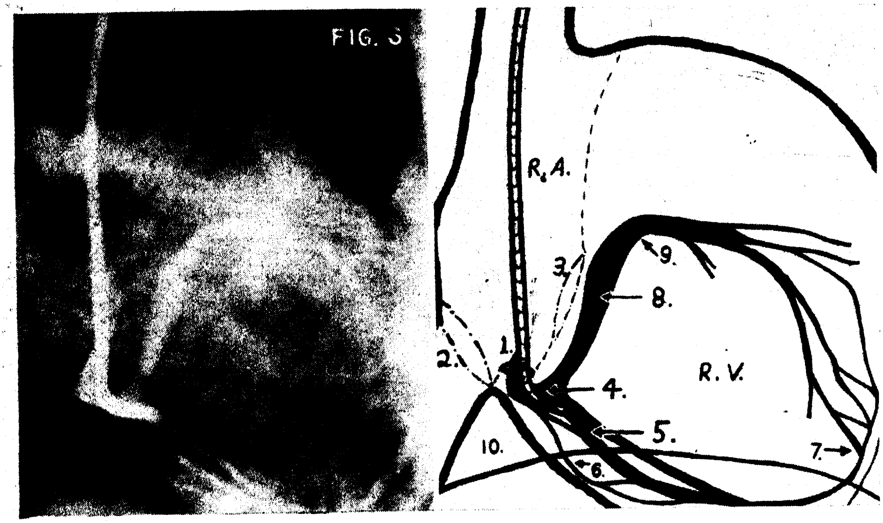


FIG. 6. CATHETER OBSTRUCTING THE FIRST POSTERIOR VEIN of the left ventricle. Diodrast injection outlines many of the veins draining into the coronary sinus through veno-venous anastomoses. 1. coronary sinus ostium; 2. inferior vena cava; 3. tricuspid valve; 4. coronary sinus *position 1*; 5. first posterior vein of the left ventricle; 6. middle cardiac vein; 7. apical veno-venous anastomoses; 8. coronary sinus *position 3*; 9. great cardiac vein, with valves illustrated; 10. lung triangle.

There is a constriction of the sinus at the first valve and a sharp decrease in caliber of the great cardiac vein as it swings down the anterior longitudinal sulcus. This vein drains the anterolateral surface of the left ventricular myocardium and also receives branches from the left auricle.

In summary, diodrast venograms and autopsy studies indicate that a nonoccluding catheter, inserted only one to two cm. inside the auricular ostium of the coronary sinus (position 1 or 2, fig. 1A or 1B), should collect representative samples of coronary venous blood draining almost entirely from the left ventricular myocardium. If this position of the catheter tip allowed withdrawal of blood from the middle cardiac vein, a significant proportion of the right ventricular myocardial flow might be obtained. Gregg has emphasized, however, that the anterior cardiac veins, which do not drain into the coronary sinus, are the major drainage channels of the right ventricle (11). The physiological role of the Thebesian veins is still disputed, (11, 12). Because of the sharp decrease in venous caliber at the opening of the great cardiac vein, however, varying degrees of venous obstruction may occur when the catheter is inserted too far, as already noted in vivo from pulse pressure recordings.

D. *Hazards of coronary sinus catheterization and other catheterization procedures in dogs.* In 48 survival experiments, recovery was uniformly prompt, without any clinical complications attributable to catheterization. Postoperative care included one dose of penicillin in beeswax and oil, 300,000 units i.m., 0.4 grams of ferrous sulfate daily and exercise with return to routine diet as early as 12 hours after the experiment.

Autopsy studies, however, often showed endocardial damage when the dogs were sacrificed following catheterization of not only the coronary sinus but also the right auricle and pulmonary artery, (35). A more controlled study is now in progress, but the findings incidental to the development of coronary sinus catheterization technique may be summarized as follows:

In a recent series of 28 dogs, autopsied within 10 days after catheterization, only three dogs were free of lesions at autopsy. Six had minimal lesions consisting of small subendocardial hemorrhages and tiny mural thrombi. The remainder had moderate to severe lesions of varying sizes, consisting of mural thrombi and subendocardial hemorrhages which sometimes extended well into the myocardium. These lesions occurred along the course of insertion of the catheter, including the tricuspid and pulmonary valves after catheterization of the pulmonary artery.

Six autopsies three to six weeks following catheterization showed small patches of minimal subendocardial fibrosis in three cases; one of these showed a small, well-organized thrombus in the superior vena cava. There was one case of marked fibrosis of the medial tricuspid valve leaflet, possibly unrelated to catheterization. There were no lesions in two cases.

In these dogs, varying degrees of difficulty in catheterization were encountered. Catheters ranging from sizes 6 to 10 were used, usually #7 or 8. The catheter was left in place from zero to five hours. Aseptic technique, omission of heparin administration and use of a small catheter did not consistently eliminate the occurrence of endocardial lesions, whether the catheter was inserted into the coronary sinus, pulmonary artery or only the right auricle.

This series was not well enough controlled to draw definite conclusions, but endocardial lesions were usually minimal when extreme care was taken to avoid trauma on insertion and when the catheter was left in place no more than one and one half hours.

Deep catheterization of the coronary sinus, however, to position 3 or beyond (fig. 1C), often caused lesions peculiar to this procedure alone. There were three cases of gross myocardial hemorrhage in areas drained by the catheterized vein, particularly in the mitral valve and apical areas. In two additional cases of deep prolonged insertion, there was a well organized thrombus occluding the great cardiac vein. These lesions have been consistently avoided in 16 recent cases using definite precautions, including gentle insertion of a #7 catheter only one to 2 cm. inside the sinus.

Electrocardiographic studies. Limb and precordial electrocardiograms were taken before, during and after coronary sinus catheterization in 34 experiments. In three out of 11 dogs in which the catheter was inserted into the great cardiac vein, (position 3 or beyond, fig. 1C), T-wave inversions and S-T segment elevations were noted following the procedure, in one or more leads, which suggested pericardial (or epicardial) irritation. Such changes were associated at subsequent autopsy in one case with thrombotic occlusion of the great cardiac vein and in another with hemorrhage of the myocardium beneath the epicardium, in areas drained by the catheterized vein. No electrocardiographic changes were noted following 23 experiments in which the catheter was inserted only one to 2 cm. inside the coronary sinus.

TABLE 3. CONTROL VALUES OBTAINED BY REPEATED CATHETERIZATIONS OF THE SAME ANIMAL

(male dog—weight 31 kgm.)

DATE		CORONARY FLOW	O^2 CONSUMP.	ART. O_2	VEIN O_2	MABP	CARDIAC RATE
		cc/100G/min.	*cc/100G/min.*	*vol. %*	*vol. %*	*mm Hg*	
6/17/47	#1	64.5	8.65	15.4	2.0	135	155
	#2	58.0	8.18	16.3	2.2	147	160
7/ 8/47	#1	90.5	12.4	17.0	3.3	142	135
	#2	77.0	10.5	16.6	2.9	146	130
7/29/47	#1	55.5	8.34	18.5	3.5	142	140
8/19/47	#1	79.0	9.80	16.5	4.1	142	160
	#2	71.0	10.12	18.0	3.7	116	156
Average.....		70.8	9.71	16.9	3.1	138	148

Continuous recordings on Lead II during insertion of the catheter into the coronary sinus, among 37 experiments, showed only occasional isolated nodal extrasystoles in 32 cases, and one case of transient nodal tachycardia lasting 20 seconds. Insertion of the catheter through the tricuspid valve, however, invariably produced very rapid bursts of ventricular and nodal extrasystoles. Similar extrasystoles were sometimes seen when the catheter tip hit the right ventricular wall below the pulmonary conus.

DISCUSSION

Endocardial damage from catheterization of the heart by the present technique has not been reported previously in dogs or man, except for one case presented by Johnson (36). This patient had congenital heart disease with cyanosis and polycythemia, and at autopsy showed multiple thrombi along the course of insertion of the catheter one month following the procedure. In several large series, however, covering over a thousand catheterizations by several investigators, and including numerous autopsied cases, there has been no apparent damage to the heart from catheterization procedures in man, (6, 15, 23). The fact that a catheter often causes endocardial lesions in dogs, but apparently not in man, may be related to differences in technique, or more likely, to species differences in the response to injury. For example, the general tendency to blood clotting and postoperative intravascular thrombosis has been commonly observed to be greater in dogs than in man (37). However, thrombi in dogs were seldom present less than 12 hours after catheterization, or more than three weeks after catheterization, so that healing apparently occurred within three weeks. Lesions in man resulting from catheterization thus might not be found unless autopsy were performed within a comparable period. It seems unlikely that insertion of the catheter through the external jugular vein can be the determining factor, or that this approach is much more traumatic than the brachial approach used in man. Anesthesia may in some way influence the development of lesions. Tachycardia under nembutal anesthesia, and the poor fixation of the heart in the dog's mediastinum, may increase the friction between catheter and endocardium. The size of these hearts, however (90–240 grams), is well within the range of the hearts of children and some adults.

In conclusion, deep insertion of a catheter three cm. or more into the coronary sinus or great cardiac vein in dogs often appears to be unduly traumatic and undesirable because of coronary venous obstruction. This conclusion is based upon a correlation of anatomical and pathological studies with electrocardiographic and pressure recordings in vivo. A recent series of experiments, presented in the accompanying report (18), have yielded satisfactory coronary flow measurements with the catheter inserted only one to 2 cm. inside the sinus ostium (fig. 1A or 1B) with minimal damage from the procedure and with no evidence of contamination of blood samples with auricular blood. Therefore, definite precautions, including gentle insertion of a small catheter only a short distance inside the coronary sinus ostium, may well be advisable in applying this technic to similar studies in man. With these precautions in the dog, however, coronary sinus catheterization appears to be no more traumatic than catheterization of the pulmonary artery.[1]

SUMMARY

1. A technic of coronary sinus catheterization in intact dogs has been presented. Modifications in the catheter design and the optimal position of the dog for fluoroscopic control of insertion have been discussed. The procedure has permitted repeated observations of coronary blood flow and myocardial metabolism in the same dogs over a period of months.

2. A high rate of cardiac utilization of oxygen, lactate and pyruvate, with a correspondingly high carbon dioxide production, were consistently found in the normal dog. Glucose utilization was relatively lower and highly inconstant.

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[1] The occurrence of endocardial lesions in dogs following catheterization of the pulmonary artery has been confirmed recently by Hellems, Haynes, Fanger and Dexter (38). The lesions were similar, but occurred with less frequency and severity than in the present series.

3. Although technical refinements have minimized the occurrence and size of lesions, it should be realized that endocardial damage cannot yet be entirely avoided in dogs following catheterization of not only the coronary sinus but also the pulmonary artery.

4. Peculiar to coronary sinus catheterization, however, were the coronary venous thromboses or myocardial hemorrhages which sometimes followed prolonged insertion of a catheter far into the coronary sinus or a cardiac vein. The elevated pulse pressure, 9 to 20 mm. Hg, found on deep insertion, indicated a significant degree of coronary venous obstruction by the catheter.

5. Coronary venous and myocardial damage were avoided by definite precautions, including gentle insertion of a small catheter only one to two cm. inside the coronary sinus. In this position there was no evident admixture of coronary blood samples with auricular blood, evidence of trauma to the auricle or coronary sinus ostium was minimal and pulse pressures were the same or only slightly higher than in the auricle, indicating that there was no significant coronary sinus obstruction. Similar precautions may well be desirable in further applications of coronary sinus catheterization.

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REFERENCES

1. Goodale, W. T., M. Lubin and W. G. Banfield, Jr. Am. J. Med. Sci. **214:** 695, 1947.
2. Kety, S. S. and C. F. Schmidt. This Journal **143:** 53, 1945.
3. Eckenhoff, J. E., J. H. Hafkenschiel, C. M. Landmesser and M. H. Harmel. This Journal **149:** 634, 1947.
4. Eckenhoff, J. E., J. H. Hafkenschiel, M. H. Harmel, W. T. Goodale, M. Lubin, R. J. Bing and S. S. Kety. Am. J. Med. Sci. **214:** 694, 1947.
5. Sosman, M. C. and L. Dexter. Radiology **48:** 441, 1947.
6. Bing, R. J., L. D. VanDam, F. Gregoire, J. C. Handelsman, W. T. Goodale and J. E. Eckenhoff. Proc. Soc. Exp. Biol. Med. **66:** 639, 1947.
7. Burwell, C. S., and L. Dexter. Trans. Assoc. Am. Physicians **60:** 59, 1947.
8. Thompson, R. H. S. and R. E. Johnson. Biochem. J. **29:** 694, 1935.
9. Harrison, T. R., B. Friedman and H. Resnick, Jr. Arch. Int. Med. **57:** 927, 1936.
10. Morawitz, P. and A. Zahn. Zentr. Physiol. **26:** 465, 1912.
11. Gregg, D. E. Physiol. Revs. **26:** 28, 1946.
12. Ratnoff, O. D. and M. Plotz. Medicine **25:** 285, 1946.
13. Forssman, W. Klin. Wochschr. **8:** 2085, 1929.
14. Cournand, A. and H. A. Ranges. Proc. Soc. Exp. Biol. Med. **46:** 462, 1941.
15. Cournand, A. Federation Proc. **4:** 207, 1945.
16. Schmidt, C. F. and S. S. Kety. Trans. Assoc. Amer. Physicians **60:** 52, 1947.
17. Kety, S. S., B. D. Polis, C. S. Nadler and C. F. Schmidt. J. Clin. Invest. **26:** 1186, 1947.
18. Eckenhoff, J. E., J. H. Hafkenschiel, M. H. Harmel, W. T. Goodale, M. Lubin, R. J. Bing and S. S. Kety. This Journal **152:** 356, 1948.

19. Sendroy, J., Jr. J. Biol. Chem. **91:** 307, 1931.
20. Bing, R. J., C. B. Thomas and E. C. Wapples. J. Clin. Invest. **24:** 513, 1944.
21. Seligman, A. M., H. A. Frank and J. Fine. J. Clin. Invest. **25:** 1, 1946.
22. Wiggers, H. C. This Journal **140:** 519, 1944.
23. Dexter, L., F. W. Haynes, C. S. Burwell, E. C. Eppinger, R. E. Seibel and J. M. Evans. J. Clin. Invest. **26:** 554, 1947.
24. Kinney, F. D., F. W. Haynes and L. Dexter. J. Lab. Clin. Med. **30:** 1013, 1945.
25. Kety, S. S. and C. F. Schmidt. To be published.
26. Roughton, F. H. W. and P. F. Scholander. J. Biol. Chem. **148:** 541, 1943.
27. Van Slyke, D. D. and J. B. Neill. J. Biol. Chem. **61:** 523, 1924.
28. Nelson, N. J. Biol. Chem. **153:** 375, 1944.
29. Somogyi, M. J. Biol. Chem. **160:** 61, 1945.
30. Friedemann, T. E. and Haugen, G. E. J. Biol. Chem. **147:** 415, 1943.
31. Barker, S. B. and W. H. Summerson. J. Biol. Chem. **138:** 535, 1941.
32. McGinty, D. A. and A. T. Miller. This Journal **103:** 712, 1933.
33. Evans, C. L., A. G. DeGraff, T. Kosaha, K. Mackenzie, G. E. Murphy, T. Vacek and O. H. Williams. J. Physiol. **80:** 21, 1934.
34. Himwich, H. E., Y. D. Koskoff and L. H. Nahum. Proc. Soc. Exp. Biol. Med. **25:** 347, 1928.
35. Goodale, W. T., M. Lubin, J. E. Eckenhoff, J. H. Hafkenschiel and W. G. Banfield, Jr. Proc. Soc. Exp. Biol. Med. **66:** 571, 1947.
36. Johnson, A. L., D. G. Wollin and J. B. Ross. Can. Med. Assoc. J. **56:** 249, 1947.
37. Conley, C. L. and W. I. Morse, 2nd. Am. J. Med. Sci. **215:** 158, 1948.
38. Hellems, H. K., F. W. Haynes, H. Fanger and L. Dexter. Personal communication.

MEASUREMENT OF CORONARY BLOOD FLOW BY THE NITROUS OXIDE METHOD[1]

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The nitrous oxide method introduced by Kety and Schmidt (1) for the quantitative measurement of cerebral blood flow in man is applicable in principle to any organ from which a representative sample of venous blood can be collected. The heart, by reason of its essentially homogenous structure and venous drainage into the coronary sinus (predominately from the left ventricle), should be a suitable organ. The applicability of the nitrous oxide method to the coronary circulation of the dog was established by a set of experiments begun more than two years ago but hitherto reported only in preliminary form (2, 3). The potential value of this procedure was greatly enhanced by the recent development of a method for catheterizing the coronary sinus (4), which makes possible quantitative estimations of coronary blood flow and cardiac oxygen consumption in the dog under conditions differing from normal only by the presence of light anesthesia, and even in intact man, once the validity and safety of the procedure have been established. The question of safety has been considered in the preceding paper (4). This report presents data bearing on the validation of the nitrous oxide method for measuring blood flow as applied to the coronary circulation of the dog.

The experiments were of two main types: *a*) those in which the indirect nitrous oxide method was calibrated against simultaneous direct measurement of blood flow by means of a bubble flowmeter; and *b*) those in which the nitrous oxide method was used alone but with variations intended to test its validity and to reveal the best procedure to employ.

I. COMPARISON OF BLOOD FLOW DATA OBTAINED SIMULTANEOUSLY BY THE BUBBLE FLOWMETER AND NITROUS OXIDE METHODS

Method. The method used to compare with the nitrous oxide technic was that of the bubble flowmeter (3, 8). Dogs, weighing 15 to 23 kgm. were anesthetized with pentobarbital sodium (30 mgm. per kgm. intravenously with 30 mgm. supplements if needed). We attempted to keep anesthesia minimal. Heparinized blood was circuited from a cannulated right carotid artery through the flow-

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[2] National Research Council Fellow in Anesthesiology.

[3] Captain, M.C., A.U.S.

meter and thence into a cannulated anterior descending branch of the left coronary artery. The chest was closed, the pneumothorax was reduced, and the animal allowed to resume spontaneous respirations. Bubble flowmeter and nitrous oxide measurements were made simultaneously.

The technic of the nitrous oxide method of measuring blood flow. This was accomplished by the method of Kety and Schmidt (1, 9). In the early experiments, a 40 per cent nitrous oxide and 60 per cent oxygen mixture was used, preceded by a 20 minute period of denitrogenation with 100 per cent oxygen. In the more recent experiments, a mixture containing 15 per cent nitrous oxide, 21 per cent oxygen and 64 per cent nitrogen was used without a period of denitrogenation.

A pair of respiratory, low-resistance flutter valves was attached to the tracheal cannula. The inflow was from a four-liter rubber bag supplied from the tank of nitrous oxide mixture. Precautions were taken to avoid leaks and the possibility of rebreathing the gas mixture.

Before the nitrous oxide technic can be applied to a particular tissue, the partition coefficient of nitrous oxide in that tissue should be known. This was accomplished by *in vitro* experiments using dog and human hearts. The method used was the same as that used by Kety, Harmel, Broomell and Rhode in establishing a partition coefficient for nitrous oxide in cerebral tissue (10). In brief, dog or human hearts were homogenized in a Waring Blender, placed in a tonometer and allowed to come to equilibrium with nitrous oxide gas. The tissue was then analyzed for the nitrous oxide content. The results are summarized in table 1.

The source of blood samples 1. *Venous.* In the first eight experiments, coronary venous blood was obtained from the coronary sinus by means of a catheter inserted through the right auricular appendage and into the sinus a maximum of 2 cm. The results of these experiments have been briefly reported elsewhere (3). Because 6 of the 15 measurements of coronary blood flow indicated gross contamination, we abandoned this source of venous blood. As will be seen below (Table 4), no such contamination occurs when the catheter is introduced from inside the venous system and is free to move with the heart. At the time, however, it seemed advisable to obviate all risk of contamination with blood from the right auricle, and for this purpose a silver cannula was tied into the great cardiac vein and the blood was allowed to flow continuously into a small flask. This blood was returned to the animal at frequent intervals via the femoral vein. Only the experiments in which venous blood was obtained in this fashion have been included in the comparison of the bubble flowmeter and the nitrous oxide methods.

2. *Arterial.* Arterial blood samples must be withdrawn from the efferent limb of the bubble flowmeter. If this is not done, the venous concentrations of nitrous oxide develop abnormally slowly in relation to the arterial concentrations. The explanation is that arterial blood to the area being studied must pass through the bubble meter before reaching the coronary circulation, the transit requiring an average of one minute. If the arterial samples are not withdrawn from the efferent limb of the meter, then the sampling time is one minute earlier than the time

the blood enters the myocardium. This factor has led to difficulties in investigations of the cerebral circulation (9, 11).

Care of the blood syringes. Blood samples were withdrawn into oiled syringes, the tips of which were filled with heparin. Arterial, coronary venous and mixed venous samples were collected synchronously. The syringes were then capped, immediately immersed in ice water and placed in a refrigerator until the bloods were analyzed for nitrous oxide content (1). Blood samples for oxygen and carbon dioxide determinations were treated in the same manner and were analyzed by the method of Van Slyke and Neill.

RESULTS. A simultaneous comparison of the nitrous oxide and bubble flowmeter methods of measuring coronary blood flow has been made 17 times in 10 experiments. The average results are shown in table 2. In general, there was a satisfactory agreement between the two methods.

TABLE 1. HEART: BLOOD PARTITION COEFFICIENT OF NITROUS OXIDE

	NO. ANALYSES	AVG. COEFFICENT	STANDARD ERROR
Dog	5	1.05	0.013
Human	5	1.13	0.013

TABLE 2. COMPARISON OF MEAN VALUES FOR CORONARY BLOOD FLOW MEASURED SIMULTANEOUSLY BY THE BUBBLE FLOWMETER AND NITROUS OXIDE METHODS
(17 determinations in 10 experiments)

BUBBLE FLOW-METER	NITROUS OXIDE	COEFFICIENT OF CORRELATION	PARTITIÓN COEFFICIENT
cc/100G/min.	*cc/100G/min.*		
63.7	67.8	0.77	0.97 s.e. ±0.052

An analysis of the data (table 2) indicates that the flows obtained were not identical. Several factors may have contributed to this discrepancy. (*a*) There may have been an overlap of arterial supply to the area of the heart whose venous drainage was being studied. In order to obtain complete agreement between the two technics, all blood in the venous sample must be supplied through the bubble meter. The importance of the site of collection of the arterial samples mentionéd before indicates that arterial blood in the area under measurement reached a given concentration of nitrous oxide one minute (more or less depending on the rate of flow) after arterial blood which did not pass through the meter. (i.e. in intact coronary arteries). If these two arterial sources overlapped, the resultant venous nitrous oxide concentration would rise faster than it should and the nitrous oxide flow would be too rapid.

This overlap unquestionably existed in many instances. In only a few experiments did temporary occlusion of the arterial inflow cause cessation of the venous outflow. Also, we became convinced that the venous drainage of the heart was too widespread to say that any one vein drains only a specific area supplied by a specific artery. In bubble meter experiments performed with the chest open, we have injected a dye (Evans Blue) into the efferent tube of the meter and have seen it appear in all the veins visible on the heart, right and left ventricular wall alike. Prinzmetal, *et al.* (12) have obtained more precise results on this subject.

(*b*) Our method of establishing blood flow per hundred grams of left ventricle per minute (8) may have led to errors. The procedure used was to terminally inject a solution of Evans Blue dye into the efferent tube of the meter and sacrifice the animal before recirculation could occur. The dyed portion of the heart was then carefully cut away from the unstained portion and weighed. The metered flow was considered as flow to this area and on this basis flow per hundred grams of left ventricle per minute was established. Overstaining, understaining or poor separation of dyed and undyed areas are all possibilities.

In view of the overlap mentioned, it is surprising that the agreement between the two methods was so close. The results therefore indicated that the nitrous oxide method was capable of yielding valid measurements of coronary blood flow and should receive a trial in the intact animal.

Since the constant of partition as determined from the bubble meter experiments was 0.97 and that from the *in vitro* dog heart experiments was 1.05, we felt that a value of 1.0 could reasonably be assigned to this coefficient.

II. MEASUREMENT OF CORONARY BLOOD FLOW IN THE INTACT DOG BY THE NITROUS OXIDE METHOD ALONE

Since the two methods showed satisfactory correlation when used simultaneously, we were ready to proceed with estimations of coronary blood flow by the nitrous oxide method with the newly developed procedure for catheterizing the coronary sinus of the intact dog (4) as soon as the latter became available. Because of the contamination encountered in the bubble flowmeter experiments (presumably with blood from the right auricle) when a catheter was introduced by direct exposure into the coronary sinus (3), we sought at first to have the catheter tip penetrate as far as possible into the coronary vernous system. For this purpose we used large dogs and advanced the tip of the catheter to a point just inside the great cardiac vein, meaning that the tip was at least 3–4 cm. from the ostium of the coronary sinus. As a final precaution we withdrew the coronary blood samples at a uniform rate calculated to be less than that at which blood was entering the coronary veins, i.e. no faster than 6 cc. in 30 seconds.

These precautions were taken in all the following experiments.

Methods. In ten experiments, the coronary sinus was catheterized by the method previously described (4). A slow flow of normal saline was allowed to pass through the catheter. Mean arterial blood pressure was recorded from a femoral artery by a mercury manometer. In all but three experiments the animals were allowed to recover.

The nitrous oxide was administered through an endotracheal tube. Passage of air around the tube was prevented by an inflated balloon on the tube or by carefully packing the pharynx. The respiratory flutter valve and the nitrous oxide administration were as described before. Blood samples were withdrawn through manifolds (see (9)) connected to the femoral artery and coronary sinus catheters.

Results. The mean control values of ten experiments in which coronary blood flow was measured 17 times are summarized in table 3. It can be seen that by this method coronary blood flow per hundred grams of left ventricle was slightly above the value previously reported from control values obtained with the bubble flowmeter on another group of experiments. The oxygen consumption figure was correspondingly slightly higher. The lower coronary venous oxygen content in these experiments was probably a reflection of the lower arterial oxygen content. It is of note that the coronary arterio-venous oxygen difference was approximately the same in both groups.

In the course of these experiments we came to believe that the contamination observed in the early experiments probably did not mean inevitable admixture of blood from the right auricle with the sample collected from the catheter in the coronary sinus. Reasons for this belief are given below (see Discussion). Since insertion of the catheter into the coronary veins was found to increase the incidence and severity of pathological changes in the myocardium (see 4), shallow insertion would be preferable if it could be proved equal to deep insertion from the standpoint of collection of uncontaminated coronary venous blood. Accordingly two final groups of experiments were made in which the catheter tip was *a*) not more than 2 cm. and *b*) 4–5 cm. inside the ostium. The results are summarized in table 4. In these experiments the shallow insertion led to significant contamination in only 2 out of the 10 cases as compared with 5 out of 7 of the deeper insertions. The values for coronary flow and cardiac oxygen consumption were significantly higher in the shallow insertion than in the deeper ones. However, a very recent series of 4 cm. insertions has yielded values comparable with those obtained in these animals with 2 cm. insertion, and this difference at least may turn out to have been fortuitous. At present it seems proper to conclude only that the position of the tip of the catheter is immaterial

TABLE 3. COMPARISON OF MEAN CONTROL VALUES OBTAINED SEPARATELY BY THE NITROUS OXIDE AND THE BUBBLE FLOWMETER METHODS

	NITROUS OXIDE		BUBBLE FLOWMETER*	
	Control values	Coeff. var.	Control values	Coeff. var.
No. experiments	10		10	
No. observations	17		19	
Coronary flow (cc/100G/min.)	71.3	15.7	66.0	13.0
Oxygen consumption (cc/100G/min.)	9.5	19.1	8.8	13.0
Art. O_2 content (vol. per cent)	16.4	11.5	19.0	13.2
Cor. venous O_2 cont. (vol. per cent)	2.9	25.1	5.7	44.0
Mean art. B.P. (mm. hg)	129	9.1	124	19.0
Cardiac rate	156	11.8	175	20.8

* (3)

so long as it is well within the coronary sinus and does not obstruct the flow in any of its tributaries.

DISCUSSION. In analyzing this method of measuring coronary blood flow, several important questions arise.

1. Many of the curves drawn from the data indicate some evidence of 'contamination'. By 'contamination' is meant the failure of the venous curve to approach the arterial as a single exponential function. Two primary factors could be involved here, either working in combination or separately: *a*) Admixture of coronary venous blood with mixed venous blood and *b*) the presence of another tissue which has a slower blood flow and/or a greater nitrous oxide capacity than cardiac muscle, viz. fat.

Mixed venous blood could come from either retrograde flow into the coronary sinus or from retrograde flow through Thebesian channels from either the right or left ventricle as suggested by the work of Prinzmetal, *et al.* (12). If retrograde flow occurred from either of these sources, one would expect the degree of contamination to vary widely from animal to animal. This has not been the case. In the experiments summarized in table 3, the degree of 'contamination', as determined by the nitrous oxide curves, amounted to an average of 4.4 per cent with a range of 0 to 14.5 per cent. Even in the experiments summarized in table 2 where the venous blood was obtained only from the great cardiac vein, thus ruling out retrograde flow from all sources but Thebesian channels from the left ventricle, there was an average of 3.0 per cent 'contamination'.

In an endeavor to obtain data of a more unequivocal nature, we have attempted several experiments in which samples from the coronary sinus catheter were withdrawn during the injection of an 0.1 per cent solution of Evans Blue Dye into the inferior vena cava or into the right auricle. In none of these experiments was there any evidence of gross contamination of the coronary venous blood, but the method was not delicate enough to give evidence as to the source of the 4.5 per cent 'contamination' noted with the nitrous oxide flows. We believe that the absence of variability from animal to animal, the evidence obtained by the dye injection, and the infrequency of contaminated curves from shallow insertion of the catheter is strong enough to indicate that the 'contamination' is not from the admixture of coronary venous blood with other venous blood.

If one assumes therefore, that it is tissue other than cardiac muscle, probably fat, that causes the 'contamination', then the blood flow values as reported herein are probably somewhat less than true coronary flow. It may be possible, by what promises to be a fairly complex mathematical analysis (13), to resolve the compound curves into their pure components. Until such time, however, the relative insignificance of the factor of 'contamination' permits our values to represent a satisfactory approximation of true myocardial blood flow.

2. A second question of major importance is, does the myocardium come into equilibrium with the arterial nitrous oxide content by the end of ten minutes? We were unable to devise a method of administering nitrous oxide to a dog and then obtaining anerobic samples of the myocardium for analysis. In investigating this factor as applied to the brain, Kety *et al.* (10) found that the brain did

come into equilibrium with the cerebral venous blood within ten minutes. On comparing the capillary density of the myocardium and the brain (see Craigie, 14, for the brain and Wearn, 15, for the myocardium) it was found that the myocardium is approximately five times more vascular than the brain; thus the heart should reach equilibrium even more rapidly than the brain.

3. When one applies this method to the measurement of coronary blood flow, is the result a measure of coronary blood flow for the entire heart or does it apply to a specific area? Venous blood appearing in the coronary sinus is primarily an outflow from the left ventricle (16, 17). However, apparently considerable amounts of blood from other sources may appear in the sinus (12). While we have made no measurements of blood flow in the right coronary artery nor of the oxygen consumption of the right ventricle, there seems to be no reason why these two factors should behave differently than they do in the left ventricle. However, due to the work differential, rate of flow and oxygen utilization by the right ventricle may differ from that which is occurring simultaneously in the left ventricle. This was well shown by the work of Moe and Visscher (18). The admixture of the blood in the coronary sinus from right and left ventricle is probably not sufficient for the sinus blood to represent a true average of all coronary venous blood. Thus we prefer to consider the figures obtained from the nitrous oxide flows as representing left ventricular blood flow.

TABLE 4. COMPARISON OF MEAN VALUES OBTAINED WHEN CORONARY VENOUS BLOOD WAS COLLECTED 2 CM AND 4 OR MORE CM INSIDE THE OSTIUM

	4+ CM		2 CM	
	Control values	Coeff. var.	Control values	Coeff. var.
No experiments	6		7	
No observations	7		10	
Coronary flow cc/100G/min.)	74	7.5	86	11.0
Oxygen consumption (cc/100G/min.)	9.1	8.0	12.3	17.0
Art. O_2 content (vol. per cent)	16.8	14.0	18.0	7.0
Cor. venous O_2 content (vol. per cent)	4.2	25.0	3.6	26.0
Mean art. B.P. (mm Hg.)	126	13.0	134	11.0
Cardiac rate	134	16.0	145	8.0

Difficulties and limitations of the method as applied to the heart. 1. Occasionally after the catheter has been introduced into the coronary sinus, it is not possible to get a free withdrawal of blood. The apparent explanation for this difficulty lies in the structure of the coronary sinus and the veins opening into it. At the opening of each major vein into the coronary sinus there are valves of the semilunar type (19). The valve that guards the opening of the great cardiac vein into the coronary sinus lies at the end of the sinus and thus directly in the path of the catheter should it be introduced 3 cm. or more. If the catheter tip gets into the pocket of this valve, it cannot be readily advanced nor can blood be withdrawn from it since the valve flap effectively occludes the tip. Careful manipulation of the catheter may remove it from this pocket, or if the tip of the catheter has multiple openings, blood may be withdrawn.

The sharp curvature of the sinus may interfere to some extent if the catheter is not sufficiently flexible. Here, the tip of the catheter impinges against the wall of the sinus and withdrawal of blood becomes difficult. Again, a catheter with multiple openings at the tip is useful.

2. If the catheter is advanced too far into the great cardiac vein or if the catheter is too large, a complete or partial obstruction of the venous return into the coronary sinus might occur. A marked pulsation of the saline in the Murphy Drip attachment to the catheter suggests this complication. Such an obstruction would not only make nitrous oxide determinations unreliable but would also invalidate the oxygen and carbon dioxide findings. Using large dogs, medium-size catheters (size 7 or 8F) and by advancing the tip of the catheter about 2–4 cm. into the sinus as determined by fluoroscopic vision, we could usually obviate this difficulty.

3. Trauma to the coronary sinus system has been noted in our series. This has been discussed in the preceding paper (4) and needs no further comment here.

4. The nitrous oxide method of measuring blood flow can be applied only if the conditions under which the flow is measured remain static for at least the ten minutes required for measuring the flow. This decreases the value of the method for the study of the action of many comparatively shorter-acting drugs.

Subject to these limitations, we believe that the nitrous oxide method of measuring coronary blood flow is practicable. The method has yielded results comparable to those obtained by the bubble meter in the dog. In view of the untoward pathological findings in the dog, the adaptation of this method to man must be made with caution. However, one cannot overlook the fact that the coronary sinus of man has been catheterized many times incidental to right auricular catheterization (5–7), and there has been as yet no indication that the procedure is dangerous.

SUMMARY

The nitrous oxide method of measuring blood flow has been investigated to determine its adaptability to the coronary circulation. The method has been calibrated by simultaneous bubble flowmeter determinations. Control values for measurements of the coronary blood flow of the dog by this method are presented and compared with similar control values obtained previously with the bubble flowmeter. The difficulties and limitations of the method in its application to the coronary blood flow are discussed. Untoward effects have become less frequent and severe as our experience and skill have increased but they have not been eliminated by any refinements thus far developed.

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REFERENCES

1. Kety, S. S., C. F. Schmidt. This Journal **143**: 53, 1945.
2. Eckenhoff, J. E., J. H. Hafkenschiel and C. M. Landmesser. Am. J. Med. Sc. **213**: 123, 1947.
3. Eckenhoff, J. E., J. H. Hafkenschiel, C. M. Landmesser and M. H. Harmel. This Journal **149**: 634, 1947.
4. Goodale, W. T., M. Lubin, J. E. Eckenhoff, J. H. Hafkenschiel, and W. G. Banfield, Jr. This Journal, **152**: 340, 1948.
5. Bing, R. J., L. D. Vandam, F. Gregoire, J. C. Handelsman, W. T. Goodale and J. E. Eckenhoff. Proc. Soc. Exp. Biol. and Med. **66**: 239, 1947.
6. Sosman, M. C. Radiology **48**: 441, 1947.
7. Wilkins, R. To be published.
8. Eckenhoff, J. E., J. H. Hafkenschiel and C. M. Landmesser. This Journal **148**: 582, 1947.
9. Kety, S. S. and C. F. Schmidt. J. of Clin. Invest., in press.
10. Kety, S. S., M. H. Harmel, H. T. Broomell and C. B. Rhode. J. Biol. Chem., in press.
11. Schmidt, C. F., S. S. Kety and H. H. Pennes. This Journal **143**: 33, 1945.
12. Prinzmetal, M., B. Simkin, H. C. Bergman and H. E. Kruger. Am. Ht. J. **33**: 420, 1947.
13. Kety, S. S. and H. N. Laden. To be published.
14. Craigie, E. H. Circulation of the Brain and Spinal Cord, Vol. XVIII, Assoc. for Research in Nerv. and Ment. Dis. Williams & Wilkins, 1938.
15. Wearn, J. T. J. Exp. Med. **47**: 273, 1928.
16. Gregg, D. E., R. E. Shipley and T. G. Bidder. This Journal **139**: 732, 1943.
17. Katz, L. N., K. Jochim and W. Weinstein. This Journal **122**: 252, 1938.
18. Moe, G. K. and M. B. Visscher. This Journal **125**: 461, 1939.
19. Spalteholz, W. Hand Atlas of Human Anatomy, 7th Ed. Lippincott, vol. II, p. 402.

NORMAL HUMAN ARTERIAL OXYGEN SATURATION DETERMINED BY EQUILIBRATION WITH 100 PER CENT O_2 IN VIVO AND BY THE OXIMETER[1]

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Until recently, the normal range for the saturation of arterial hemoglobin with oxygen has been considered to be 93 to 97 per cent. In 1944, Roughton, Darling and Root (1) analyzed critically certain errors that may occur in the determination of the percentage saturation of hemoglobin with oxygen and concluded that the average normal figure of 95 per cent should be revised upward to 97 per cent. At the same time, Drabkin and Schmidt (2) measured the arterial oxygen saturation in five normal males by a spectrophotometric technique and found an average figure of 98.6 per cent with a very narrow range (98.0 to 99.3 per cent).

Because of the great importance of the estimation of arterial oxygen saturation as an overall test of lung function, we decided to perform further studies upon a larger group to determine *a*) what constitutes normal arterial oxygen saturation and *b*) whether presumably normal individuals vary widely about an average figure or whether normality is confined to very narrow limits.

Roughton, Darling and Root (1) concluded that the errors in the Van Slyke technique lay not in the determination of O_2 *content* of the arterial blood, but in the estimation of the O_2 *capacity* by the in vitro equilibration technique. We decided, therefore, to avoid the errors inherent in the in vitro equilibration technique and to determine O_2 capacity by an in vivo equilibration technique employing known concentrations of O_2 in the alveoli. After determining the content of O_2 in the arterial blood while the subject was breathing room air, the capacity of hemoglobin to take up O_2 was found by measuring the arterial O_2 content during inhalation of 100 per cent O_2. Appropriate corrections were made in each case for the amounts of O_2 present in the bloods in physical solution. An example follows: If the O_2 content of arterial blood taken from a subject during inhalation of room air was 19.3 vols. per cent and the O_2 content of arterial blood taken during inhalation of 100 per cent O_2 was 22.04 vols. per cent, when appropriate subtractions are made to eliminate the O_2 dissolved in physical solution in the bloods at the respective O_2 tensions (3), these figures become 19.0 and 20.0 vols. per cent. The saturation of arterial blood (during inhalation of room air) thus becomes 19/20 or 95 per cent.

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[1] The work described in this paper was done under contract between the Medical Division, Chemical Corps, U. S. Army, and the University of Pennsylvania. Under the terms of the contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the authors.

This method of 'in vivo equilibration' with 100 per cent O_2 to determine O_2 capacity of arterial blood was carried out in a series of 20 normal males, and the oxygen saturations calculated as above. In addition, for purposes of comparison, arterial oxygen saturations were determined in the same subjects by *a*) the usual technique of determining capacity by in vitro equilibration of blood with room air and *b*) noting the rise in oximeter readings of arterial oxygen saturation upon breathing 100 per cent O_2.

METHODS

A. *General.* Twenty normal males between the ages of 18 and 63 served as subjects; 12 were normal medical students and the remainder were ambulatory patients with no known pulmonary disease. Each subject lay quietly in bed for 15–20 minutes. During this time an oximeter was placed upon his ear and sufficient time allowed for full vasodilatation of the ear vessels. The oximeter was set to read 95 per cent saturation. The subject breathed through valves, and the minute volume of respiration was recorded by conducting the expired air into a balanced compensated spirometer equipped for graphic recording. The region around a brachial or femoral artery was thoroughly infiltrated with 1 per cent procaine. A 10 cc. syringe with a #20 gauge intraspinal needle attached was prepared by drawing a small amount of heparin solution into the syringe, pulling the plunger back to the 10 cc. mark so that the walls of the syringe were wet with heparin. The air and the remainder of the heparin were then slowly ejected through the needle. In this way the dead space in the needle and syringe (average 0.15 cc.) was filled with anticoagulant. The needle was then inserted into the artery; in no instance did the subject experience discomfort or know the precise time of arterial puncture. None of the respiratory records showed hypo- or hyperventilation during the withdrawal of blood. Ten cc. were withdrawn, the syringe was disconnected from the needle and the latter was styletted and left in place. The syringe was immediately capped, taken to a Van Slyke manometric gas analyzer prepared to receive it and the analysis for CO_2 and O_2 content was begun at once (blood #1).

As soon as the first blood sample was withdrawn the subject was given 100 per cent O_2 to breathe for 10–15 minutes. This period was considered sufficiently long to produce maximal arterial oxygen levels because *a*) inhalation of 100 per cent O_2 reduces the alveolar air (and presumably the arterial blood) nitrogen to negligible values in five minutes and *b*) oximeter records demonstrate that the arterial oxygen saturation reaches its peak within 2–3 minutes after inhalation of 100 per cent O_2. After 10–15 minutes, a second sample of arterial blood was withdrawn and immediately analyzed for CO_2 and O_2 content (blood #2).

B. *Van Slyke technique.* The same 1.0 cc. pipette was used for duplicate analyses. In order to prevent any loss of blood gases during the filling and emptying of the pipette, a modified mercury-filled pipette was used in *experiments 11 to 20* so that the blood was never exposed to air. Before each analysis the blood was mixed thoroughly by agitating with a small amount of chemically clean mercury that had been drawn into the syringe. Two or three analyses were performed on each sample, the blood being stored in the capped, iced syringes between analyses. The standard error in the series of duplicate determinations was 0.07 per cent; the standard error for the calculated O_2 saturation was 0.5 per cent saturation.

Each sample was also analyzed in the Evelyn photo-electric colorimeter for total hemoglobin pigment after conversion to cyanmethemoglobin. This was done to detect any change in red blood cell-plasma ratio that might have occurred in the circulating blood between the withdrawal of *samples 1* and *2*. In 9 of the 20 subjects, the readings were identical in the two samples; in the others slight corrections were necessary.

Calculations were performed as follows. *Blood # 1:* The oxygen contents obtained in the duplicate analyses were averaged, and 0.3 cc. subtracted from this figure; 0.3 cc. represents the amount of O_2 physically dissolved in arterial blood at 38° C. (3) at the pO_2 of 100 mm. Hg believed to represent average arterial oxygen in normal subjects breathing room air (4, 5). The figure thus obtained represents the amount of O_2 combined with hemoglobin in blood #1. *Blood # 2:* Duplicate analyses were averaged. If the total pigment, as determined by the determination of cyanmethemoglobin, differed in bloods #1 and #2, the O_2 content of blood #2 was corrected to that both samples represented the same amount of total pigment. Then 2.04 cc. were subtracted; 2.04 cc. represent the amount of physically dissolved O_2 in arterial blood at 38°C. and a pO_2 of 673 mm. Hg. (That the figure of 673 mm. Hg may be too high will be commented upon later.) The resulting figure represents the amount of O_2 combined with hemoglobin in blood #2. The fully corrected figure for blood #1 divided by that for blood #2 represents the arterial O_2 saturation of blood #1 (that obtained during breathing room air).

This method was checked by an in vitro equilibration technique which was performed as follows: A portion of blood #1 was rotated in a 50 cc. flask for five minutes at room temperature. Then two pipettes were filled simultaneously in order to obtain blood of the same red blood cell-plasma ratio. One of these was used for analysis for O_2 capacity in the Van Slyke apparatus (blood #3) and the other for determination of total pigment after conversion to cyanmethemoglobin. The value obtained for O_2 capacity was corrected in two ways: *a)* 0.56 to 0.63 cc. was subtracted to correct for the amount of physically dissolved O_2 in blood at 154 mm. pO_2 and at temperatures ranging from 29° to 24° C.; *b)* if the total pigment differed in bloods #1 and #3, corrections were made to equalize the total pigments. In 12 of 17 cases the total pigment was greater in the equilibrated sample, thus supporting Roughton's statement that drainage errors in the glass vessel cause the sample to be unduly rich in red blood cells (1). O_2 saturation was then calculated as: $\frac{\text{corrected blood content}}{\text{corrected blood capacity}} \times 100 =$ per cent O_2 saturation.

C. *Oximeter technique.* After an ear 'warm-up' period of 15 minutes and an instrumental 'warm-up' period of 45 minutes, the oximeter (6) was set to 95 per cent saturation when the subject was breathing room air. The maximal increase that followed inhalation of 100 per cent O$_2$ was noted (within two to five minutes after blood #1 was withdrawn). The O$_2$ saturation of the subject during breathing of room air was considered to be 100 per cent minus the per cent saturation *increase* that occurred during O$_2$ inhalation. A similar procedure was carried out in 39 additional subjects on whom oximetry alone was done, and no arterial samples were withdrawn. In all 58 cases, ear thickness readings were noted frequently to be certain that conditions were steady throughout the experimental period.

TABLE 1. CORRECTED O$_2$ CONTENTS OF ARTERIAL BLOODS TAKEN DURING INHALATION OF ROOM AIR AND 100% O$_2$

SUBJECT #	[1]CORRECTED CONTENT		PER CENT SATURATION	O$_2$ ADDED TO HEMOGLOBIN	SUBJECT #	[1]CORRECTED CONTENT		PER CENT SATURATION	O$_2$ ADDED TO HEMOGLOBIN
	room air	100% O$_2$				room air	100% O$_2$		
				cc.					*cc.*
1	18.45	19.71	93.6	1.26	11	17.50	17.86	98.0	0.36
2	16.40	16.66	98.4	0.26	12	21.25	22.51	94.4	1.26
3	19.80	20.19	98.1	0.39	13	17.45	18.16	96.1	0.71
4	19.25	20.13	95.6	0.88	14	17.00	17.46	97.4	0.46
5	19.75	19.66	100.5	−0.09	15	18.87	19.13	98.6	0.26
6	18.75	18.99	98.7	0.24	16	16.70	17.28	96.6	0.58
7	16.90	17.06	99.1	0.16	17	15.32	15.82	96.8	0.50
8	21.25	21.99	96.6	0.74	18	17.50	17.56	99.7	0.06
9	20.40	20.54	99.3	0.14	19	18.63	18.43	101.0	−0.20
10	18.05	18.66	96.7	0.61	20	18.15	19.09	95.1	0.94

[1] Corrected *a*) by subtraction of amount of physically dissolved O$_2$.; *b*) by equilization of total hemoglobin pigments in the two samples.

TABLE 2. COMPARISON OF O$_2$ SATURATIONS ESTIMATED BY THREE METHODS

SUBJECT #	IN VIVO TECH.	IN VITRO TECH.	OXIMETER TECH.	SUBJECT #	IN VIVO TECH.	IN VITRO TECH.	OXIMETER TECH.
1	93.6	—	94.5	11	98.0	94.7	96.0
2	98.4	—	98.0	12	94.4	96.4	95.0
3	98.1	98.4	97.5	13	96.1	95.2	97.0
4	95.6	97.5	96.5	14	97.4	—	94.0
5	100.5	98.8	95.0	15	98.6	95.7	96.0
6	98.7	99.2	97.0	16	96.6	98.5	95.5
7	99.1	97.0	97.0	17	96.8	95.0	97.0
8	96.6	98.8	95.0	18	99.7	101.4	98.0
9	99.3	97.2	96.5	19	101.0	100.0	95.0
10	96.7	98.1	97.0	20	95.1	93.2	—
Average....................................					97.5	97.4	96.2

RESULTS

In table 1 are shown the corrected O$_2$ contents of 20 normal basal individuals breathing room air and 100 per cent O$_2$. On the basis of these figures, the O$_2$

saturation of the arterial blood averaged 97.5 per cent, with a range of 93.6 to 101.0 per cent. Eight of 19 determinations varied from the average by more than 2 S.E. of the method.

In table 2 is shown a comparison of the O_2 saturations determined in these subjects by the three methods. The average figures for the 'in vivo' and 'in vitro' saturations show excellent agreement; the individual differences may be accounted for largely by a summation of experimental errors in the determination of oxygen content and total pigment. The average saturation determined by the oximeter technique was only 96.2 per cent.[2] The standard error of the mean of the differences between the 'in vivo' equilibration and the oximeter in the 19 cases was 0.5 and the *t* value was 3.0, so that the difference is clearly significant.

DISCUSSION

Several possible sources of error in our methods should be considered. First of all, the absolute values for O_2 saturation depend upon the estimation of dissolved O_2 in the two blood samples. The amount of dissolved O_2 depends upon the arterial pO_2 and the α O_2 at 38°C. The arterial pO_2 was not measured but estimated. The arterial pO_2 of normal males breathing room air at rest is known to be about 100 mm. Hg (4, 5), and a small variation here would not alter the results appreciably. On the other hand the arterial pO_2 during inhalation of '100 per cent' O_2 may be less than 673 mm. Hg for several reasons: *a*) the O_2 used contains only 99.7 per cent O_2; *b*) small amounts of N_2 must enter the alveolar air from the venous blood during the experimental period; and *c*) the arterial pO_2 may be 10 to 30 mm. below the alveolar pO_2 for a variety of reasons (7, 8). If the arterial pO_2 were as much as 30 mm. below the alveolar pO_2, this would tend to reduce the arterial saturations (calculated by the in vivo equilibration technique) by approximately 0.5 per cent.

We have used in this study the average figure for α O_2 at 38°C. determined for whole ox blood by Sendroy, Dillon and Van Slyke (3). It is possible that this varies slightly from species to species and from individual to individual. No allowances have been made for this.

While the in vivo equilibration technique is useful experimentally, it is not applicable to clinical usage, for it can be employed only when the arterial pO_2 is measured or can with reasonable accuracy be estimated. In patients with cardiorespiratory disease, large errors may occur if arterial pO_2 is assumed during inhalation of either room air or of 100 per cent O_2.

It appears from this study, as from a previous investigation (1), that the in vitro equilibration technique for O_2 saturation can be made more accurate by correcting for drainage errors and for dissolved O_2 (3). Many clinical laboratories measuring O_2 saturation by the in vitro technique fail to make either correction. Failure to make the simple correction for dissolved O_2 results in a figure for O_2 saturation of 1.5 per cent below the true value, if the equilibration was carried out at room temperature.

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[2] Dr. W. M. Boothby of the Mayo Foundation determined arterial oxygen saturation by the oximeter technique in a large group of normal men and found it to be approximately 97 per cent (personal communication).

It should also be pointed out here that the error is such in the determination of O_2 saturation by manometric techniques that significance should not be attached to small differences.

The error appears to be greater in the use of the oximeter. First of all, the average saturation was significantly lower by this technique. In addition, oximeter increases of 5 per cent occurred in *subjects 5* and *19*, both of whom were completely saturated by one or both of the other techniques. While this may be explained as a summation of errors, it is possible that the oximeter scale may be incorrect in the 95–100 range (expanded too much) or that the oximeter may be analyzing blood that contains slight admixtures of venous blood (either because of incomplete capillary dilatation, because of O_2 consumption by the ear tissues, or because of vasoconstriction induced by oxygen inhalation).

We have employed the oximeter increase on breathing O_2 as a rough measure of the original arterial O_2 saturation. The limitations of this are obvious: the oximeter measures *changes* and not *absolute* saturations, unless the oximeter is first set at a known value. In our experience with 58 normal subjects, inhalation of O_2 raised the oximeter reading an average of 3.8 with a S.D. of 1.36 and a range of 2 to 6 per cent. Thus an increase upon breathing O_2 of more than 8 per cent (average + 3 S.D.) indicates definite anoxemia. The reverse is not true, i.e. a change of less than 8 per cent does not necessarily rule out anoxemia. In our experience, however, the *rate* of increase of arterial O_2 saturation is a more sensitive indicator of cardiorespiratory abnormality than is the *amount* of increase (9).

The results obtained in this study by the in vivo and in vitro techniques agree with the findings of Roughton *et al.* (1) that average normal arterial O_2 saturation is greater than 95 per cent and closer to 97.5 per cent. The latter figure is 1.0 to 1.5 per cent lower than that obtained by Drabkin and Schmidt (2). The variability in our subjects cannot be explained wholly on the basis of the experimental errors listed. It is possible *a*) that a lower average arterial O_2 saturation occurs in some individuals because of improper mixing or diffusion of gases in or through circulated alveoli or because of other shunts or *b*) that some methemoglobin or carbon monoxide hemoglobin exists in the arterial blood of certain individuals; these may be converted into O_2 carrying hemoglobin in the presence of a high arterial pO_2. Methemoglobin concentration was not determined in this study. Van Slyke *et al.* (10) found an average of only 0.4 per cent methemoglobin in freshly drawn venous blood and noted that in about half the bloods its concentration was so low as to be uncertain. The mean total 'inactive' hemoglobin was found to be 1.3 per cent in their subjects. Van Slyke did not determine these values upon arterial blood; it is possible that 'inactive' hemoglobin would be less or absent in arterial blood, as indicated by the work of Drabkin and Schmidt (2).

The blood of *subject 20* was tested for carbon monoxide hemoglobin by a very sensitive method by Dr. Spealman and none could be found. Since the blood of this subject was 95.1 per cent saturated, this low figure could not have been accounted for by the presence of carbon monoxide hemoglobin in blood #1 and its absence in blood #2 of the in vivo equilibration technique. No other tests for COHb were made, but all of the smoker-subjects (with one exception) had not smoked for several hours to several days before the tests.

SUMMARY

The arterial oxygen saturation was determined in 20 subjects by an in vivo equilibration technique which avoids the chief errors encountered by the usual in vitro technique.

Normal arterial oxygen saturation by this technique averaged 97.5 per cent.

Saturations were measured in the same subjects by a corrected in vitro equilibration technique and by the oximeter. The former yielded an average figure of 97.4 per cent and the latter, 96.2 per cent. The usefulness of and errors in these techniques are discussed.

REFERENCES

1. Roughton, F. J. W., R. C. Darling and W. S. Root. This Journal **142:** 708, 1944.
2. Drabkin, D. L. and C. F. Schmidt. J. Biol. Chem. **157:** 69, 1945.
3. Sendroy, J., R. T. Dillon and D. D. Van Slyke. J. Biol. Chem. **105:** 597, 1934.
4. Comroe, J. H., Jr. and R. D. Dripps. This Journal **142:** 700, 1944.
5. Lilienthal, J. L., Jr., R. L. Riley, D. D. Proemmel and R. E. Franke. This Journal **147:** 199, 1946.
6. Millikan, G. A. Rev. Sci. Inst. **13:** 434, 1942.
7. Berggren, S. M. Acta Physiol. Scand. **4:** 1, 1942, Supplement XI.
8. Fasciolo, J. C. and H. Chiodi This Journal **147:** 54, 1946.
9. Fowler, W. S. and J. H. Comroe, Jr. J. Clin. Invest. In press.
10. Van Slyke, D. D., A. Hiller, J. R. Weisiger and W. O. Cruz J. Biol. Chem. **166:** 121, 1946.

STUDIES OF THE PULMONARY CIRCULATION AT REST AND DURING EXERCISE IN NORMAL INDIVIDUALS AND IN PATIENTS WITH CHRONIC PULMONARY DISEASE[1, 2]

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Studies of the cardiac output of normal individuals during muscular exercise have been made by several investigators (1–5). Systemic blood pressure has likewise been measured during exercise, making possible the calculation of peripheral resistance and the work of the left ventricle. Until recently (5), such information was not available in the case of the right ventricle because pulmonary arterial pressure was not known. By using the technic of pulmonary artery catheterization, both cardiac output and pulmonary arterial pressure can be determined during exercise, and pulmonary resistance and the work of the right ventricle can be calculated. This report deals with such studies on three normal individuals and eight patients with chronic pulmonary disease from whom data were obtained at rest and during exericse.

In some patients with chronic pulmonary disease the pulmonary arterial pressure and cardiac output at rest are normal (6, 7), and it is of interest to determine whether the pulmonary arterial pressure becomes abnormally high during exercise. In these patients and in others who show high pulmonary arterial pressure at rest it is of importance from the point of view of estimating their disability to determine whether there is abnormally low cardiac output or abnormally high work of the right ventricle during exercise.

METHODS

In most instances the subjects were given mild sedation on the night before and the morning of the studies and came to the laboratory without breakfast. The normal subjects took no sedation and had orange juice, black coffee, and dry toast for breakfast at least an hour and a half before the first samples were taken.

The catheter was introduced through the basilic vein or one of its tributaries with the subject lying on a horizontal fluoroscope, and the tip of the catheter was placed in the truncus of the pulmonary artery (8–11). An indwelling arterial needle was placed in the brachial artery on the other side. Resting pulmonary

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[1] Under grants from the Commonwealth Fund and the Life Insurance Medical Research Fund.

[2] W. F. Hamilton and J. W. Remington, Department of Physiology, University of Georgia School of Medicine, collaborated on a comparison of cardiac output methods in which some of the data herein presented were used.

and brachial artery pressures were recorded with a Hamilton manometer and samples were taken for cardiac output determination. The subject then stood up, walked across the room, and seated himself on a bicycle ergometer. During the transfer the saline infusion through the catheter was continued by holding the flask high, and the arm with the arterial needle was kept extended. In spite of slight motion of the arms and head during exercise, no great difficulty was encountered in withdrawing blood samples, collecting expired air or recording pulse-pressure curves.

All exercise measurements were taken in a 'semi-steady' state, i. e., after six or seven minutes of exercise at a constant rate and after the pulmonary ventilation had assumed an approximately constant value. The work load was varied by applying a brake and by changing the rate of pedaling, but was calibrated only on the basis of the subject's oxygen consumption in ml. per minute. Pulmonary and brachial artery pulse-pressure tracings during exercise were taken with an electrical type of manometer (12). The zero point for pulmonary pressure measurements was taken as the third intercostal space anteriorly. Pulmonary arterial pressure recordings taken with the subject seated on the bicycle before exercise served as controls and were comparable to pressures measured in the supine position. The tracings showed more artefacts during exercise than at rest because of motion of the catheter, and therefore the values for systolic and diastolic pressure were somewhat less accurate than the corresponding values at rest. Mean pressures, calculated by planimetric integration of the pulse-pressure curves throughout several complete respiratory cycles, were probably not in error by more than 2 mm. Hg.

Vascular resistances in the pulmonary circuit and in the greater circuit were calculated according to the formulae:

$$R \text{ (pulmonary)} = \frac{P.A._m \times 1.332 \times 60}{C.O.}$$

$$R \text{ (peripheral)} = \frac{B.A._m \times 1.332 \times 60}{C.O.}$$

where R = vascular resistance in dynes-sec.-cm.$^{-5}$
$P.A._m$ = mean pressure in the pulmonary artery in mm. Hg
$B.A._m$ = mean pressure in the brachial artery in mm. Hg
C. O. = cardiac output in l. per minute.

Since neither the left nor the right atrial pressure was known, neither was introduced into the expression for pressure drop in the numerator of the equations. Atrial pressure is close to zero in the absence of heart failure or valvular disease, however, and any error resulting from the omission of this factor was not great in the cases under consideration. There is some evidence that the mean pressure in the left auricle is a few mm. Hg higher than in the right (13), and therefore resistance in the pulmonary vascular bed as calculated may tend to be overestimated.

The conventional formulae were used to make a rough approximation of the work of the ventricles against pressure:

$$W \text{ (right ventricle)} = C.\,O. \times P.\,A._{m} \times 0.1332$$

$$W \text{ (left ventricle)} = C.\,O. \times B.\,A._{m} \times 0.1332$$

The work of the ventricles is more accurately determined by using arterial pressures during systole rather than over the entire cardiac cycle. When this is done, the results come out 10 to 40 per cent higher, according to a recent publication by Remington and Hamilton (14), because the mean pressure during systole is 10 to 40 per cent greater than the mean pressure over the whole of the cardiac cycle. We have not calculated ventricular work in the approved manner because of the extreme irregularity of the pulse-pressure tracings during exercise which would have necessitated an arbitrary decision as to the duration of systole. Since we will be concerned with relative rather than absolute values for the work of the right and left ventricles, the conventional formulae are believed to provide an adequate estimate of work done against pressure.

In making physiological interpretations we should like to know how closely work done against pressure approximates the total work of the ventricles. It is therefore necessary to know the order of magnitude of kinetic work. Authorities agree that it is insignificant at rest, but since it varies as the cube of the volume flow of blood and inversely as the fourth power of the radius of the vessel into which the blood is ejected (15), any change in these factors occurring during exercise must be reflected many times over in the value for kinetic work. Accurate information regarding the radii of the pulmonary artery and aorta during work is not available, and the effect of various chronic pulmonary diseases upon the distensibility of these vessels is difficult to estimate during life. Sample calculations for normal subjects suggest that kinetic work constitutes approximately 3 per cent of the total work of the right ventricle at rest and that the percentage increases considerably during heavy exercise (15, 16). The kinetic work of the left ventricle is approximately the same as that of the right in absolute terms but constitutes a much smaller percentage of total left ventricular work. Disease states which narrow the pulmonary artery or reduce its distensibility must increase the kinetic work of the right ventricle.

MATERIAL FOR STUDY

The three normal subjects were physicians in good health, and the eight patients suffered from various types of chronic pulmonary disease. Their physical characteristics and diagnoses are shown in table 1.

RESULTS

Normal subjects. In table 2 the data obtained on the normal subjects are presented and the average values for rest and two grades of exercise are calculated. For the milder exercise the oxygen consumption varied from 269 to 512 ml. per minute per square meter of body surface; for the more severe work the range was

TABLE 1

CASE NO.	DIAGNOSIS	AGE	SEX	HT.	WT.	B.S.A.
				cm.	*kgm.*	*sq. m.*
377	Normal	36	M	176	62.8	1.78
378	Normal	32	M	178	66.6	1.84
379	Normal	26	M	178	70.5	1.87
366	Left pneumonectomy, partial thoracoplasty, for tuberculous bronchial stenosis	34	F	150	48.0	1.41
371	Chronic pulmonary tuberculosis, left fibrothorax, re-expanded pneumothorax	34	F	160	65.8	1.71
372	Nodular pulmonary fibrosis, cause undetermined; chronic pulmonary emphysema, moderate	39	F	160	48.5	1.48
383	Silico-tuberculosis, moderate extent	39	M	181	84.0	2.05
354	Post-traumatic left fibrothorax, mediastinal dislocation	44	F	160	66.0	1.69
368	Chronic pulmonary emphysema, pulmonary fibrosis healed tuberculosis	40	M	165	59.0	1.65
358	Saccular bronchiectasis, recent acute bronchopneumonia with mild decompensation	54	M	178	74.5	1.92
344	Thrombosis of the left pulmonary artery, mild cardiac decompensation	41	F	154	47.0	1.42

TABLE 2

NO.	STATE	HEART RATE	O_2Hb SATURATION (%)	OXYGEN CONSUMPTION (ML/MIN/M^2B.S.)	A-V DIFFERENCE (VOL %)	CARDIAC OUTPUT (L/MIN/M^2)	STROKE VOLUME (ML/BEAT)	BLOOD PRESSURES, (MM. HG)						VASCULAR RESISTANCE DYNES SEC. CM^{-5}		WORK OF THE VENTRICLES AGAINST PRESSURE		
								Brachial artery			Pulmonary artery					Left	Right	
								syst.	diast.	mean	syst.	diast.	mean	Peripheral	Pulmonary	joules/min.	joules/min.	joules/min./m^2
377	Rest	47	97	111	4.2	2.62	99	120	59	82	20	10	15	1400	249	51	9	5
	Exercise (1)	77	95	436	7.6	5.68	131	144	83	112	24	3	8	900	60	150	10	6
	Exercise (2)	105	94	700	9.2	7.53	128	152	100	130	19	0	10	780	57	230	17	10
378	Rest	91	99	147	3.8	3.83	64	122	73	94	19	8	12	1060	135	89	11	6
	Sitting	100	—	—	—	—	—	—	—	—	18	9	13	—	—	—	—	—
	Exercise (1)	127	97	512	8.2	6.17	90	133	88	108	21	5	12	835	93	160	18	10
	Exercise (2)	151	96	912	9.9	9.11	111	168	101	131	20	6	8	625	39	292	18	10
379	Rest	73	96	120	3.6	3.35	86	93	63	76	—	—	11	972	144	63	9	5
	Exercise (1)[1]	90	99	269	6.2	4.36	91	97	64	79	—	—	13	752	123	86	14	8
	Sitting	86	—	—	—	—	—	—	—	—	—	—	13	—	—	—	—	—
	Exercise (2)	157	97	824	11.6	7.14	85	140	83	107	—	—	14	634	84	190	25	13
Average of 3 normals																		
	Rest	70	97	126	3.9	3.27	83	112	65	84	20	9	13	1144	176	68	10	5
	Exercise (1)	98	97	406	7.3	5.40	104	125	78	100	23	4	11	829	92	132	14	7
	Exercise (2)	138	96	812	10.2	7.93	108	153	91	123	19	3	10	680	60	237	20	11

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[1] Leg exercise performed in the supine position.

from 700 to 912. Because of the differences in oxygen consumption per square meter, the figures for arterio-venous difference and cardiac output cannot be compared directly. In two of the three normal subjects the stroke volume increased significantly with exercise; in the third there was little change in stroke volume. There was a fall in pulmonary arterial mean pressure with inceasing work in two of the three normal subjects. The third subject showed an insignificant rise. The fall in mean pressure was due to a fall in diastolic pressure; the systolic pressure remained unchanged. When one considers that the cardiac output increased on the average two and one half times, it is obvious that a decrease in mean pressure could occur only in association with a sharp fall in pulmonary vascular resistance. As seen in the table, pulmonary vascular resistance decreased to approximately one third of its resting value during the more severe exercise. The estimated work of the right ventricle against pressure increased on the average only 10 joules per minute during exercise while the work of the left ventricle increased 170 joules per minute.

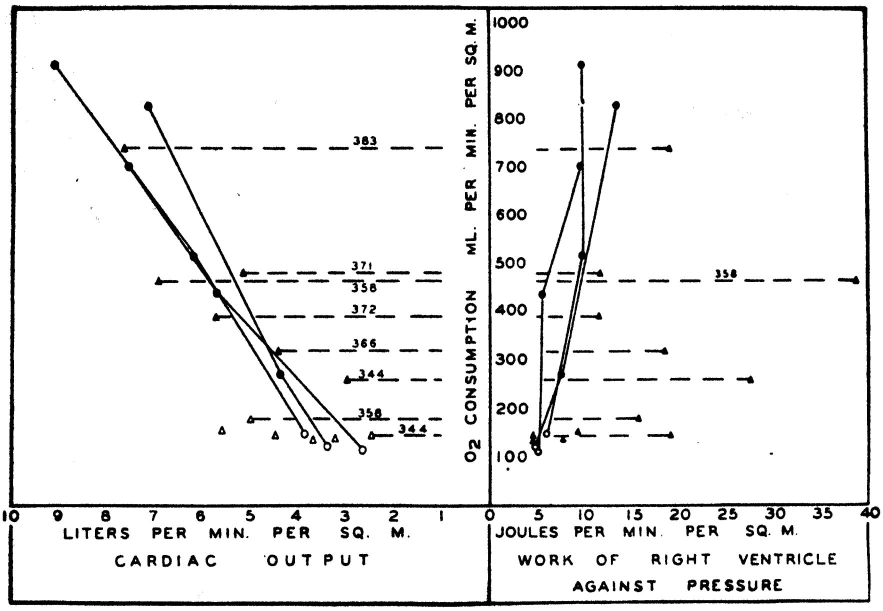

FIG. 1. RELATIONSHIPS BETWEEN OXYGEN CONSUMPTION, cardiac output, and work of the right ventricle against pressure. *Circles* represent normal subjects; *triangles* represent patients with chronic pulmonary disease; *open circles* and *triangles* represent resting values; *closed circles* and *triangles* represent values obtained during exercise; *solid lines* connect points obtained on the same normal subject at different metabolic levels; *broken lines* connect points obtained on patients during a single study.

In figure 1 the cardiac output at different levels of oxygen consumption can be studied in relation to the work of the right ventricle against pressure. The solid lines connect points obtained on the normal subjects. The almost vertical slope of the curves on the right indicates that the increase in right ventricular work against pressure was very small. There was marked similarity in the responses of the three normal individuals both with respect to cardiac output and with respect to the work of the right ventricle.

Patients with chronic pulmonary disease. The patients who were studied had a wide range of pulmonary pathology including fibrothorax, bronchiectasis, pneumonectomy, fibrosis, emphysema, silico-tuberculosis, and thrombosis of the left pulmonary artery. They were in no sense a homogeneous group, and the degree of functional disturbance varied widely. In table 3 it can be seen that the arterial oxyhemoglobin saturation in three patients (358, 344, and 368) was low at rest and decreased to a still lower level during exercise. With two exceptions (383 and 368) the severity of the work done by the patients was much less than that done by the normal subjects, as indicated by the rate of oxygen consumption.

TABLE 3

NO.	STATE	HEART RATE	O_2Hb SATURATION (%)	OXYGEN CONSUMPTION (ML/MIN/M^2B.S.)	A-V DIFFERENCE (VOL %)	CARDIAC OUTPUT (L/MIN/M^2)	STROKE VOLUME (ML/BEAT)	BLOOD PRESSURES (MM. HG)						VASCULAR RESISTANCE DYNES SEC. CM^{-5}		WORK OF THE VENTRICLES AGAINST PRESSURE		
								Brachial artery			Pulmonary artery					Left	Right	
								syst.	diast.	mean	syst.	diast.	mean	Peripheral	Pulmonary	joules/min.	joules/min.	joules/min./m^2
366	Rest	96	95	135	4.2	3.21	47	127	85	102	33	9	18	1500	318	61	11	8
	Sitting	100	—	177	4.8	3.68	52	87	60	73	31	11	20	1123	308	51	14	10
	Exercise	133	98	315	7.2	4.39	46	145	95	114	47	15	32	1476	362	94	26	18
371	Rest	108	95	132	3.5	3.67	58	132	96	112	16	6	9	1430	114	94	8	5
	Rest	111	97	147	3.1	4.59	71	118	84	102	13	6	9	1040	87	107	9	5
	Sitting	127	—	—	—	—	—	—	—	—	16	10	13	—	—	—	—	—
	Exercise	173	97	478	9.1	5.11	50	153	105	121	—	—	17	1[illegible]0	159	141	20	12
372	Rest	93	96	142	3.2	4.44	71	114	63	88	16	4	9	1070	103	77	7	5
	Sitting	104	—	—	—	—	—	—	—	—	17	7	12	—	—	—	—	—
	Exercise	129	93	387	6.8	5.70	65	140	79	101	23	11	15	965	142	113	17	12
383	Rest	132	97	151	2.7	5.55	87	126	97	108	—	—	13	754	98	164	19	9
	Sitting	120	94	149	4.1	3.63	62	—	—	—	20	13	16	—	167	—	15	7
	Exercise	183	95	736	9.6	7.65	86	174	96	126	26	14	19	1356	94	263	39	19
	Rest	136	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—	—
354	Rest	109	96	—	—	—	—	—	—	—	20	10	16	—	—	—	—	—
	Exercise	144	—	—	—	—	—	—	—	—	41	24	30	—	—	—	—	—
	Rest	141	93	—	—	—	—	—	—	—	32	11	20	—	—	—	—	—
368	Rest	83	93	142	4.1	3.48	69	124	73	92	24	4	13	1280	183	70	9	5
	Sitting	98	91	172	4.6	3.75	63	127	82	104	28	14	19	1350	240	85	15	9
	Exercise	159	86	983	—	—	—	199	111	148	59	27	43	—	—	—	—	—
358	Rest	79	91	177	3.6	4.97	120	120	60	86	33	17	23	722	182	109	30	16
	Sitting	88	—	183	5.3	3.50	76	124	60	89	32	16	23	1063	275	79	21	11
	Exercise	110	81	460	6.7	6.90	120	—	—	—	61	31	42	—	253	—	74	39
344	Rest	101	87	141	5.8	2.44	34	135	80	101	96	36	58	2330	1330	47	27	19
	Exercise	145	82	256	8.7	2.95	29	147	98	116	117	(50)	(70)	2210	(1330)	65	(39)	(28)

Because of the reduced exercise tolerance of most of the patients, it was impossible to study them at higher work levels. In the patients with chronic pulmonary disease the stroke volume either remained unchanged or decreased during exercise. Six patients (366, 371, 372, 383, 354, 368) had pulmonary arterial pressures which were normal or close to normal at rest. In three of these (371, 372, 383) the increase in pulmonary arterial pressure during exercise was moderate. The three patients (358, 344, 368) who showed the most striking increase in pulmonary arterial pressure with exercise were the same ones whose oxygen saturation dropped sharply during exercise. Except in one case (371) there was an increase in both systolic and diastolic pressure in the pulmonary artery. In one patient (344) whose pulmonary arterial pressure was extremely high at rest, the pressure was recorded from the right ventricle but not from the pulmonary artery during exercise. Since the systolic pressures in the pulmonary artery and in the right ventricle are the same, the latter were used as a measure of pulmonary arterial systolic pressure. An estimated figure of 50 mm. Hg for the pulmonary arterial diastolic was assumed and this figure was used in the calculation of mean pressure, pulmonary vascular resistance and work of the right ventricle during exercise. In all the patients with chronic disease the pulmonary vascular resistance stayed the same or increased during exercise, in contrast to the normal subjects. During exercise the work of the right ventricle against pressure for a corresponding rate of oxygen consumption was invariably higher than in the normal subjects. In two patients (344 and 358) the discrepancy was very striking.

On the left side of figure 1 the patients' cardiac output values are plotted in relation to those of the normal subjects. Points falling to the left of the solid lines indicate higher than normal cardiac output, while those to the right indicate lower than normal output. One patient only (344) had a significantly low cardiac output and she had previously shown signs of congestive heart failure. On the right side of figure 1 all the patients' points fall to the right of the solid lines, indicating that the work of the right ventricle against pressure was abnormally high for the corresponding level of oxygen consumption. With one exception (383) the exercise performed by the patients was so light that the work of the right ventricle against pressure can be taken as an approximate measure of the total work of the right ventricle.

DISCUSSION

Comparison of the values obtained for cardiac output and oxygen consumption during exercise with those obtained by Bock, van Caulaert, Dill and others (4) shows a remarkable agreement. Their cardiac output method was a modification of the Haldane method, in which mixed venous carbon dioxide tension was estimated on the basis of an intrapulmonary equilibration procedure. For the corresponding rates of oxygen consumption, the values for two of our three normal subjects (377 and 378) invariably fall between the values of AVB and DBD. At the highest exercise level our third subject (379) corresponds to CVC. The data of Lindhard (3) obtained with the nitrous oxide method are also comparable. With normal subjects running on a treadmill, Dill, Edwards, and

Talbott (2) found the same relation of cardiac output to oxygen consumption as in the case of bicycle exercise. These data, too, correspond to ours. Stroke volume relationships show more variation because of the differences in pulse rate in response to a given amount of exercise.

Von Euler and Liljestrand (17) made observations on the pulmonary arterial blood pressure in the cat during exercise and found a moderate increase in all except one case. In a very recent note Hickam and Cargill (5) working with normal human subjects using the catheter technic report pulmonary arterial pressure essentially unchanged with increases in cardiac output up to nearly twice the resting level. The present study, undertaken without knowledge of their work, corroborates and extends this finding.

On the basis of the pulmonary arterial pressures found during exercise in normal individuals, certain changes in the pulmonary vascular bed during exercise may be postulated. If the dimensions of the pulmonary vascular bed remained unchanged during exercise, the mean pressure would rise in proportion to the increase in blood flow. The failure of the mean pressure to rise implies an expansion of the pulmonary vascular bed either by a widening of existing blood channels or by the opening of new ones or by a combination of both mechanisms. An actual decrease in pulmonary arterial pressure cannot be explained as a simple mechanical effect of increased blood flow. It suggests either that active vasodilatation occurs or that the increased negativity of intrathoracic pressure associated with deeper inspirations expands the vascular bed. The latter explanation is more consistent with current thoughts on the pulmonary circulation based on other work in this laboratory. Regardless of mechanism, there can be no doubt that a very significant expansion of the pulmonary vascular bed occurs as a normal response to exercise. If, as seems likely on hemodynamic grounds, more alveolar capillaries are opened up, the physiological advantages of such a vascular response would be seen not only in reduced work of the heart but also in increased efficiency of gaseous diffusion. This is in accord with the increased diffusion constant of the lung during exercise (18).

In studying patients with emphysema who had high pulmonary arterial pressure at rest, Hickam and Cargill (5) found a further large increase in pulmonary pressure with exercise. These findings, which are entirely comparable to those of the present study, are in striking contrast to the response to exercise of normal individuals.

Two factors are possibly of importance in explaining the rise in pulmonary arterial pressure with exercise in patients with chronic pulmonary disease. The first is the size and expansibility of the pulmonary vascular bed. Destruction of lung tissue, interstitial pulmonary fibrosis, or vascular sclerosis would be expected to reduce the number and cross section of the vessels and the expansibility of the vascular bed during increased blood flow.

The second factor is related to the effect of anoxia upon pulmonary vascular resistance. It may be seen in table 3 that the three patients whose arterial oxyhemoglobin saturation dropped significantly during exercise (358, 344, and 368) were the three whose pulmonary arterial pressures were highest during exercise. Von Euler and Liljestrand's observations on cats (17) and the findings of Motley, Cournand, *et al.* (19) on human subjects indicate a sharp rise in pulmonary arterial pressure with acute anoxia produced by breathing a gas mixture low in oxygen. Might not the elevation of pulmonary arterial pressure occurring in some of our patients be related, at least in part, to the associated anoxia? In this respect it is interesting that von Euler and Liljestrand found a decrease in pulmonary arterial pressure with pure oxygen breathing to a level below that obtaining during room air breathing, suggesting that the slight degree of anoxemia normally present produces a slight increase in pulmonary vascular resistance. This argument tempts one to conclude that in subjects whose arterial blood is unsaturated at rest, a certain elevation of pulmonary arterial pressure above the normal may be due to the effects of anoxemia upon the size of the pulmonary vascular bed. However, since the evidence rests on acute experiments with high or low oxygen breathing, the application to chronic states of anoxia occurring in pulmonary disease can only be suggested as a possibility requiring further proof. In patients who have a marked decrease of oxygen saturation during exercise, the exercise may be considered as an acute experiment in anoxia, and it seems likely that the anoxemia may cause some of the increase in pulmonary arterial pressure.

The effects of chronic pulmonary disease upon the circulation may be considered from the over-all aspect of cardiac output or from the more restricted point of view of the work of the right ventricle. In figure 1 the data are plotted from both points of view. In this limited series of patients the cardiac output was maintained at an essentially normal level in all but the most extreme instance (344) even though a greater than normal work load was thereby placed upon the right ventricle. By increased work, the right ventricle compensated for the increased vascular resistance in the pulmonary circuit, and prevented a decrease in volume flow of blood.

In their revision of Bainbridge's monograph on "The Physiology of Muscular Exercise," Bock and Dill (1) state that "the heart, as a rule, reaches the limit of its powers earlier than the skeletal muscles, and its functional capacity determines a man's capability for exertion. . . . An exaggerated response on the part of the respiratory and circulatory systems involves a useless expenditure of energy; a deficient response rapidly throws out of gear the controlling influences of the nervous system, cripples the energies of the skeletal muscles, and renders exercise ineffective or even impossible." The latter situation is probably illustrated by one of our patients (344) whose cardiac output was significantly lower than normal both at rest and during exercise (fig. 1). She was able to perform only the mildest grade of work (oxygen consumption 256 ml. per minute per square meter of body surface) yet her right ventricle worked nearly three times as hard as the right ventricles of the normal subjects during heavy exercise. It seems probable that the functional capacity of the right ventricle determined this patient's capability for exertion. Another patient with increased pulmonary vascular resistance (358) was able to work nearly twice as hard in spite of a still greater work load on his right ventricle (fig. 1). Probably the greater functional capacity of this man's right ventricle made possible his greater capacity for physical exercise.

These studies illustrate some of the difficulties involved in evaluating disability in chronic pulmonary disease. In most instances subjects are unable to exert themselves to a point where their cardiac output falls below the normal for that exercise level. Accordingly studies of the greater circulation reveal little. On the other hand studies of the pulmonary circulation during exercise reveal definite abnormalities in patients whose exercise tolerance is low. When considered in conjunction with other aspects of pulmonary function they extend the physiological evidence upon which an estimate of disability must be based.

SUMMARY

1. Studies of cardiac output and pulmonary arterial pressure were performed using the venous catheter technic in three normal individuals and in eight patients with various types of chronic pulmonary disease. Measurements were made at rest and during exercise on a stationary bicycle.
2. Two of the three normal subjects showed a decrease in the mean pressure in the pulmonary artery during exercise; all showed a marked drop in pulmonary vascular resistance and a minimal increase in the work of the right ventricle during exercise.
3. Three of the patients with chronic pulmonary disease showed a significant elevation of pulmonary arterial pressure at rest, and in all eight cases the mean pressure increased during exercise. There was either no change or an increase in the pulmonary vascular resistance during exercise, and the work of the right ventricle was invariably higher than in the normal subjects at a corresponding work level.
4. The findings indicate that the expansibility of the pulmonary vascular bed during exercise is limited in patients with chronic pulmonary disease.
5. Anoxia may contribute to the elevation of pulmonary arterial pressure during exercise in those patients whose arterial oxygen saturation falls.
6. The physiological evidence upon which an estimate of disability must be based in patients with chronic pulmonary disease is extended by studies of the pulmonary circulation during exercise.

REFERENCES

1. Bainbridge, F. A. (revised by A. V. Bock and D. B. Dill). The Physiology of Muscular Exercise. Longmans, Green & Co., 1931.
2. Dill, D. B., H. T. Edwards and J. H. Talbott. J. Physiol. **69:** 267, 1930.
3. Lindhard, J. Archiv. Ges. Physiol. (Pfluger's) **161:** 233, 1915.
4. Bock, A. V., C. vanCaulaert, D. B. Dill, A. Folling and L. M. Hurxthal. J. Physiol. **66:** 136, 1928.
5. Hickam, John B. and W. H. Cargill. Am. J. Med. **3:** 504, 1947.
6. Cournand, A. Bull. N. Y. Acad. Med. **23:** 27, 1947.
7. Hamilton, W. F. and R. L. Riley *et al.* In press.
8. Cournand, Andre and Hilmert A. Ranges. Proc. Soc. Exp. Biol. and Med. **46:** 462, 1941.

9. Cournand, A., H. D. Lauson, R. H. Bloomfield, E. S. Breed, and E. deF. Baldwin. Proc. Soc. Exp. Biol. and Med. **55:** 34, 1946.
10. Cournand, A., R. L. Riley, E. S. Breed, E. deF. Baldwin and D. W. Richards Jr. J. Clin. Invest. **24:** 106, 1945.
11. Bloomfield, Richard A., Henry D. Lauson, Andre Cournand, Ernest S. Breed and Dickinson W. Richards Jr. J. Clin. Invest. **25:** 639, 1946.
12. Motley, H. L. A. Cournand, L. Werko, D. Dresdale, A. Himmelstein and D. W. Richards Jr. Proc. Soc. Exp. Biol. and Med. **64:** 241, 1947.
13. Cournand, A., H. L. Motley, A. Himmelstein, D. Dresdale, J. Baldwin. This Journal **150:** 267, 1947.
14. Remington, J. W. and W. F. Hamilton. This Journal **150:** 292, 1947.
15. Gomez, D. Personal communication.
16. Starling's Principles of Human Physiology, Lea & Febiger, 1933, p. 773.
17. Von Euler, U. S. and G. Liljestrand. Acta Physiol. Scand. **12.** 301, 1946.
18. Lilienthal, J. L. Jr., R. L. Riley, D. D. Proemmel and R. E. Franke. This Journal **147:** 199, 1946.
19. Motley Hurley L., Andre Cournand, Lars Werko, Aaron Himmelstein, and David Dresdale. This Journal **105:** 315, 1947.

EFFECT OF BODY TEMPERATURE CHANGE ON THE CIRCULATION TIME IN THE CHICKEN[1]

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We have recently reported that an increase or decrease in the body temperature in the chicken (1) and in the turtle (2) causes a corresponding change in the arterial blood pressure. The mechanism of this relationship is in need of elucidation. Among those mechanisms which must be considered as possibly playing some rôle in this relationship are *a*) changes in the heart rate which are seen as the body temperature is varied; *b*) changes in the arteriolar and capillary responsiveness at various temperatures; and *c*) adjustments originating in the central nervous system which affect the tone of the blood vessels.

A few data which furnish partial answers to this question are already available. Thus the heart rate continues to increase during induced hyperthemia (1) even though there is usually no increase, and perhaps even a fall, in blood pressure; therefore the relationship is not entirely dependent on the changes occurring in heart rate. The peripheral arterioles show no observable changes in responsiveness with temperature change since there are no notable differences in the pressor response to epinephrine at various body temperatures (1). We have obtained evidence that the central nervous system plays an important rôle in the temperature-pressure relationship since destruction of the brain or sectioning of the cervical spinal cord in the turtle eliminates this relationship. This occurs even though the heart rate continues to change with changes in the body temperature (3).

In order to analyze the effect of body temperature change on the circulation, we felt that determination of the circulation time at various body temperatures might provide some insight into the mechanisms responsible for the observed blood pressure changes. Such information would also give presumptive evidence for changes in cardiac output.

METHODS

The chicken was used because the temperature-pressure relationship can be demonstrated most consistently in this animal. A total of 118 determinations of the circulation time was made on 12 chickens. Temperatures were measured with a mercury thermometer inserted deep into the cloaca. Blood pressures were recorded optically with the Hamilton manometer in the unanesthetized chicken according to the technique previously described by this laboratory (1).

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The animals were cooled by packing them in crushed ice, and rewarmed by radiant heat (1).

The circulation time was estimated by the time of onset of the blood pressure response to the injection into the sciatic vein of 0. 2 mgm. of acetylcholine dissolved in 0. 2cc. saline. The circulation time was figured as the time from injection into the sciatic vein until the beginning of a marked fall in blood pressure.

In all 118 trials the blood pressure fell very significantly. The beginning of this pressure drop was considered to be the time required for circulation from the site of injection into the sciatic vein, through the heart and lung circuit, and to the peripheral arterioles. A significant slowing of the heart occurred in 81 of the 118 trials. However, this slowing usually occurred after the fall in systemic pressure.

RESULTS

1. *The normal circulation time of the chicken.* In our series of 12 animals the normal circulation time as determined by the fall in blood pressure averaged 2.8 seconds ranging from 1.3 to 5.0, with a standard deviation of 0.9 seconds. These data are presented in figure 1 as points at 41–42°C. In those experiments in which the acetylcholine-induced bradycardia was observed, the time for slowing required an average of 4.5 seconds, about 1.7 seconds longer than the blood pressure effect.

TABLE 1. AVERAGE CIRCULATION TIME AT VARIOUS BODY TEMPERATURES

BODY TEMPERATURE (°C)	AVERAGE CIRCULATION TIME Seconds
45	1.2
41.5	2.8
35	4.0
30	8.2
25	12.2

2. *The effect of cooling.* In six chickens cooled from the normal body temperature of about 41.5° to about 25°C., the circulation time was increased from the normal of about 3 seconds to as much as 10 or 15 seconds at the lower temperature. This fall in the rate of circulation appeared to go step by step with the fall in body temperature, as indicated by table 1. Individual data are given in figure 1.

The time required for the onset of the bradycardia is also increased at lower temperatures so that at 25°C., the slowing of the heart was not apparent until 4 seconds or so after the fall in pressure, i. e., an average of about 16 seconds.

3. *The effect of warming.* In six chickens warmed from the normal body temperature to as high as 47°C. the rate of circulation continued to increase, with the circulation time values as low as 0.6 seconds (fig. 1). This reduction in the circulation time occurred despite the fact that the blood pressure almost always had begun to fall by the time the body temperature has been raised to

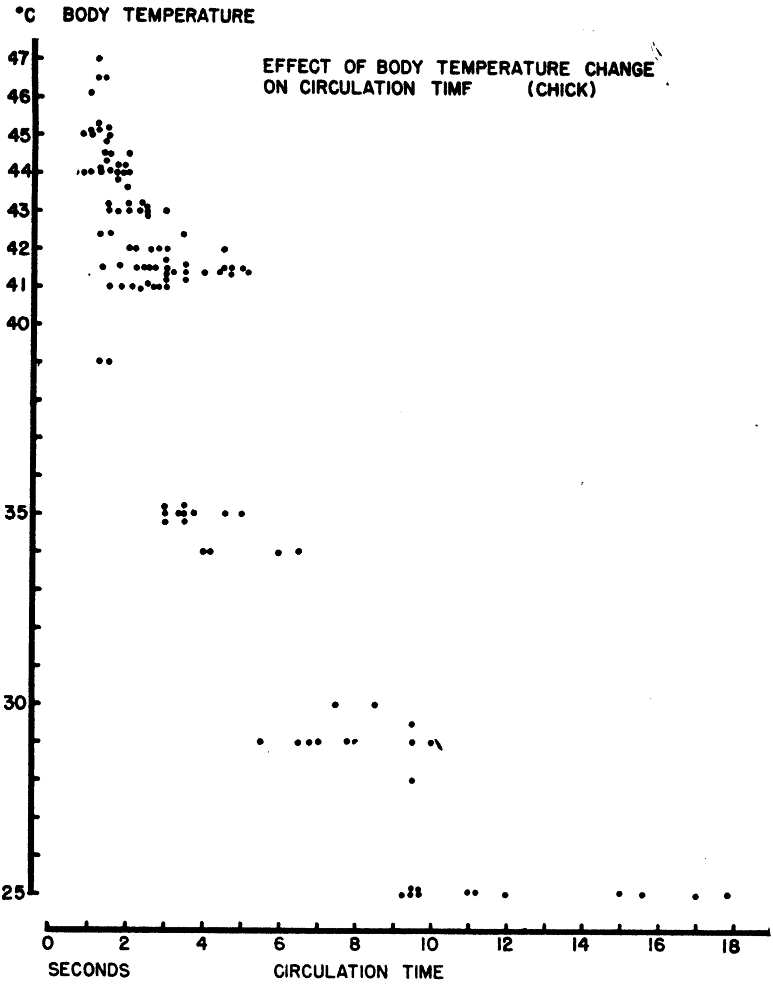

FIG. 1.

TABLE 2. RESPONSE OF BLOOD PRESSURE TO ACETYLCHOLINE INJECTIONS AT VARIOUS BODY TEMPERATURES

BODY TEMPERAURE (°C)	ARTERIAL PRESSURE	
	Pre-injection (mm. Hg)	Average minimal pressure observed mm. Hg
46	50	30
45	83	30
44	103	39
43	107	36
41.5	110	43
35	105	46
30	99	47
25	59	35

about 44°C. The heart rate also continued to increase with increasing temperatures, as previously noted (1). The bradycardia induced by acetylcholine occurred about 0.5 seconds after the depressor effect, e. g., a total time of about 1.2 seconds at 46°C.

4. *The degree of blood pressure fall.* The injection of acetylcholine lowered the pressure momentarily to levels averaging about 40 mm. Hg. The minimal level obtained was not dependent on the pre-injection level, nor was there any consistent change in the degree of the blood pressure fall as the animals were cooled or warmed (table 2.).

DISCUSSION

The normal circulation time in the chicken, 3 seconds, is quite rapid when compared with that observed with the same technique in the dog (4) or in man (5). This is probably due in part to the small size of the chicken, which reduces the length of the mean path from the point of injection to the site of action. It may also be related to the higher body temperature of this animal, and the consequent higher metabolic rate, requiring a greater circulation.

The finding that the circulation is slowed as the body temperature falls is in accord with data which we have obtained on blood pressure, heart rate (1), and with the results of Randall on respiration (6). When the animal is cooled, the reduced rate of circulation of the blood is apparently sufficient to nourish the tissues at the lower rates of metabolism which must obtain at the lower body temperatures. This may be inferred from the fact that no apparent injury occurs to the animal when, after a period of two hours at body temperatures as low as 25°C., it is rewarmed to its normal body temperature. As has been noted, the animals show no signs of shock, appear normal and resume feeding. These circumstances demonstrate that no serious disparity exists between the metabolic demands of the tissues for oxygen and substrate, and the rate of delivery of these substances to the tissues by the blood stream.

It is apparent that the circulation speeds up (decreased circulation time) as the body temperature is increased above normal and that it continues to do so practically until the point of death (7, 8). This occurs despite the fact that the blood pressure is not necessarily increased in experimental hyperthermia and that it may even fall to very low levels (65/50 mm. Hg). These results show that the circulation time is a function of the metabolic needs of the tissues rather than of the arterial blood pressure. Since the metabolic needs of the tissues depend in large part upon their temperatures, it would appear that each tissue autonomously determines the volume of blood which traverses its blood vessels.

It is of some interest that in hyperthermia in which the circulation is speeded up and the blood vessels of the tissues must be somewhat dilated to permit a greater perfusion of blood, a maximal dilatation is not obtained even at high temperatures (46°C.) since a fall in blood pressure still occurs on the injection of acetylcholine. Thus the vessels are still partially constricted under these extreme conditions.

The progressive rise in heart rate with increasing body temperatures would also appear to be an autonomous tissue function, in this case of the heart itself. It is possible that during the tachycardia seen in hyperthermia with rates of as much as 540 per minute, the heart may reach the limit of effective cardiac output and begin to show signs of failure, and this possibly may play a rôle in the death of the animals.

An unexpected finding was that the time required for the fall in pressure by action of acetylcholine on the pacemaker of the heart was considerably longer than that required for the fall in blood pressure due to the peripheral action of the drug on the arterioles. The reason for the cardiac delay is not apparent at present.

Our present data support our earlier studies in which we showed no significant change in responsiveness to epinephrine of the blood vessels at various temperatures (1). The present results are similar in that cooling or warming of the blood vessels did not significantly alter the degree of the depressor response to acetylcholine.

The reduced rate of blood flow during cooling would appear to be related not only to the greatly reduced heart rate but also to the very likely reduction in minute cardiac output. Both factors would tend to reduce the blood pressure. The likely reduction of blood vessel calibre under hypothermic conditions would tend to raise the blood pressure by increasing resistance to flow. The resolution of these conflicting trends may be a simple algebraic summation.

In hyperthermic states the dilatation produced by the increased demand for blood by the tissues would tend to cause a fall in blood pressure. The increased rate of return of blood from the tissues and the consequent increased cardiac output would tend to raise the blood pressure. However, at temperatures within two or three degrees above normal, the blood pressure regulating mechanisms appear to maintain a relatively constant level.

The regulation of the blood pressure thus appears to depend on factors other than the changes in heart rate, the circulation time or the rate of metabolism of the peripheral tissues. The blood vessels show no change in responsiveness at various body temperatures to either vasopressor and vasodepressor drugs. Instead, the mechanisms for blood pressure regulation appear to reside primarily in the central nervous system. It is these central nervous mechanisms which eventually fail as the body temperature is raised sufficiently above normal. The temperature-pressure mechanism, which functions to permit an increased perfusion of the tissues at higher temperatures, is apparently finally overwhelmed by the increasing vasodilatation in the periphery, and by approaching the limit of adequate function of the heart at high rates. Our data therefore suggest that the final failure of the circulation in extreme hyperthermia lies not only in the peripheral vessels which are dilated, but still responsive even at the extremes of body temperature, not in the ability of the heart to pump blood, but actually it depends to a very large degree upon the failure of the central nervous mechanism which acts to regulate the arterial blood pressure.

SUMMARY

1. The acetylcholine circulation time of the chicken at normal body temperatures (41.5°C.) is about 2.8 seconds.

2. When the body temperature of the animal is lowered by application of ice packs, the circulation time is increased. Increasing the body temperature with radiant heat decreases the circulation time.

3. The circulation time is not necessarily related to changes in the blood pressure which occur as the temperature of the animal is changed, but appears to be due primarily to changes in the peripheral tissues.

4. A discrepancy between the time required for the action of acetylcholine on the peripheral blood vessels and on the rate of the heart is noted.

5. Some aspects of these data are discussed in terms of the body temperature–blood pressure relationship.

REFERENCES

1. Rodbard, S. and M. Tolpin. This Journal **151**: 509, 1947.
2. Rodbard, S. and D. Feldman. Proc. Soc. Exp. Biol. Med. **63**: 43, 1947.
3. Rodbard, S. Fed. Proceedings **7**: 191, 1947.
4. Wilburne, M., J. G. Schlichter, M. Grossman and F. Cisneros. This Journal **150**: 504, 1947.
5. Schlichter, J. G. In press.
6. Randall, W. C. This Journal **139**: 56, 1943.
7. Neyman, C. A. Artificial Fever, Springfield, Ill., Charles C Thomas, 1938.
8. Altshule, M. D. and A. S. Freedberg. Medicine **24**: 403, 1945.

QUANTITATIVE STUDIES ON THE COMPARATIVE ACTIVITY OF CALCIUM AND CHEMICALLY RELATED IONS ON THE COAGULATION OF BLOOD[1]

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A direct quantitative study of the influence of varying the concentration of calcium on the coagulation time of blood was not possible until Steinberg (1) discovered that a phenol-formaldehyde resin, Amberlite IR-100, completely removed calcium without inducing any other demonstrable changes in the blood. By using chloro-methyl-silane (Silicone) coated glassware with Amberlite and by taking precautions to prevent contamination of the blood with tissue juice, the lysis of platelets is almost entirely prevented and it seems reasonable to assume that the blood obtained is practically identical with the circulating blood except for the absence of calcium. The effect of different concentrations of calcium on the clotting of blood and plasma can therefore be determined simply and with precision by the direct addition of known amounts of calcium salts. This procedure is likewise suitable for studying the action of related ions, particularly strontium, magnesium and barium.

In this paper the results of such study are reported. The influence of different concentrations of calcium, strontium, magnesium and barium on the clotting time of whole blood and plasma and prothrombin time are also considered in man, dog and rabbit. Consideration is also given to the possibility of an antagonism between calcium and related bivalent ions in the process of coagulation.

EXPERIMENTAL

Blood was obtained by venipuncture in man and dog and from the main artery of the ear in rabbits. Precaution was taken to avoid injuring tissue and to prevent foaming. Needles, syringes and collecting tubes used were coated with chloro-methyl-silane. The blood was immediately passed through purified Amberlite IR-100 according to the technique recently described (2). The collecting tube and the syringe were chilled and the decalcified blood kept in an ice bath until used.

To remove traces of calcium from the thromboplastin, sodium oxalate (0.1 ml. of 0.1 M sodium oxalate per rabbit brain) was added before triturating the brain with acetone. The chlorides of calcium, strontium, magnesium and barium were used, and their solutions were made isotonic to blood and then diluted to the desired strength by mixing with physiological sodium chloride solution; this was done to eliminate any possible influence of osmotic pressure changes. The coagulation time was determined by adding to 0.1 ml. of calcium chloride (or other salt studied), 0.4 ml. of blood or plasma decalcified with Amberlite. Pyrex tubes with an internal diameter of 11 mm. were used. All observations were carried out at 37°C.

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The effect of varying concentrations of calcium and strontium chloride on the coagulation time of blood and plasma decalcified with Amberlite. From the results recorded in table 1 it will be observed that the shortest coagulation time for human, dog and rabbit blood is not obtained until the concentration of calcium chloride is approximately 0.0015 M. This concentration is in the range of the normal calcium level of the blood, which is also about 0.0015 M. When the calcium concentration is reduced below one half of the normal, a definite delay in coagulation occurs. A minimum coagulation time is obtained over a wide range of calcium concentrations, but above 0.004 M inhibition occurs in human, dog and rabbit blood, becoming more evident as the concentration of the salt is progressively increased. Oddly, the depressing action of calcium is most marked in rabbit blood. Coagulation of plasma (table 2) follows essentially the same pattern except that the clotting time is shorter. This can be explained, at least in part, by the earlier detection of the clot in plasma than in whole blood.

TABLE 1. INFLUENCE OF THE CONCENTRATION OF CALCIUM AND STRONTIUM ON THE COAGULATION TIME OF BLOOD DECALCIFIED WITH AMBERLITE[1]

CONCENTRATION OF THE SALT[2]	COAGULATION TIME					
	$CaCl_2$			$SrCl_2$		
	Human blood	Dog blood	Rabbit blood	Human blood	Dog blood	Rabbit blood
M	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
0.0002	>60	>60	>60			
0.0005	8¾	29	27½			
0.001	6	8½	8½			
0.0015	5¾	4¼	5½	>60	>60	>60
0.002	5¾	4	5½	28[3]	>60	>60
0.004	5½	3½	5¼	12¾	19	17½
0.006	6¾	4½	8	11½	15½	12½
0.008	9	5	9¼	11	13	12¼
0.01	9	5	9½	18¾	17	17
0.012	10¼	6¼	10¼	28	39	36
0.0135	11	6¼	34	34	58[3]	>60
0.0155	11½	10½	47	59[3]	>60	
0.0175	11¾	10½	>60	>60		
0.018	13½	21				
0.02	14½	37				

[1] To four volumes of blood treated with Amberlite one volume of $CaCl_2$ or $SrCl_2$ solution was added. Isotonic solutions of these salts were prepared and diluted to the desired concentration by mixing with physiological sodium chloride solution.

[2] The molarity is calculated on basis of volume of blood.

[3] Only a few threads of fibrin formed.

Strontium has a much weaker clotting activity than calcium. The minimal level required for optimum or shortest coagulation of whole blood and plasma is definitely higher (0.006 to 0.008 M for both whole blood and plasma of man, dog and rabbit). The range of optimal concentrations at which most rapid clotting occurs is also considerably more narrow than in the case of calcium since inhibition already occurs at a level of 0.01 M (tables 1 and 2).

Magnesium is incapable of causing coagulation of either whole blood or plasma decalcified with Amberlite at any concentration. In the presence of thromboplastin, however, a weak clotting activity can be demonstrated, as will be described later. Barium is found to possess no demonstrable coagulant activity, even in the presence of thromboplastin.

TABLE 2. INFLUENCE OF THE CONCENTRATION OF CALCIUM AND STRONTIUM ON THE COAGULATION TIME OF PLASMA DECALCIFIED WITH AMBERLITE[1]

CONCENTRATION OF THE SALT[2]	COAGULATION TIME					
	$CaCl_2$			$SrCl_2$		
	Human plasma	Dog plasma	Rabbit plasma	Human plasma	Rabbit plasma	Dog plasma
M	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
0.0002	>60	>60	>60			
0.0005	7¼	12	15½			
0.001	4¼	7¼	9½			
0.0015	3¾	3¾	4	>60	>60	>60
0.002	2½	2½	2¾	28	45	>60
0.004	2	2	2½	8¾	23	29½
0.006	2½	2¾	2½	7	18½	21
0.008	3	3¼	2¾	8¼	9¾	11¾
0.01	3½	4	3	8½	11	13
0.012	5¾	6¼	6¾	8¾	15	20¾
0.0135	9	7¾	7¾	10¼	27	45
0.0155	9¼	9¾	9½	15	38	54
0.0175	19	17	17	17	58[3]	>60
0.018	21	19	22	>60	>60	
0.02	38	22	24			

[1] To four volumes of plasma decalcified with Amberlite one volume of $CaCl_2$ or $SrCl_2$ solution was added. Isotonic solutions of these salts were prepared and diluted to the desired strength by mixing with physiological sodium chloride solution.

[2] The molarity is calculated on basis of volume of plasma.

[3] Only a few threads of fibrin formed.

The comparative inhibitory action of strontium, magnesium and barium on coagulation when an optimum concentration of calcium is present. A fixed amount of calcium (0.005 M $CaCl_2$), mixed with varying concentrations of $SrCl_2$, $MgCl_2$, and $BaCl_2$, was added to decalcified plasma and the coagulation time determined. The results are summarized in table 3. It will be noted that the retarding effect of $SrCl_2$ on clotting is less marked when calcium is present and that strontium and magnesium present essentially the same inhibitory activity. This inhibitory effect is shown not only by the delayed coagulation but also by the character of the clot, which becomes softer, poorer in texture and ultimately very incomplete and almost granular. Barium, at a relatively low concentration, causes complete incoagulability of the blood. The barium ion, it should be pointed out, precipitates plasma proteins similar to other heavy metals and this probably ex-

TABLE 3. EFFECT OF DIFFERENT CONCENTRATIONS OF STRONTIUM, MAGNESIUM AND BARIUM ON THE COAGULATION TIME OF PLASMA DECALCIFIED WITH AMBERLITE IN THE PRESENCE OF AN OPTIMUM CONCENTRATION OF CALCIUM CHLORIDE (0.005 M)

CONCENTRATION OF THE SALT[1]	COAGULATION TIME					
	$SrCl_2$		$MgCl_2$		$BaCl_2$	
	Human plasma	Rabbit plasma	Human plasma	Rabbit plasma	Human plasma	Rabbit plasma
M	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
0.0004	2	2¾	2	2¾	2	2¾
0.0008	2¼	3	2	2¾	2¼	2¾
0.002	2½	3¼	2	2¾	2	3
0.004	3	3½	3	5¼	3¼	4½
0.007	3¾	4¼	4¼	8	5¼	6½
0.01	4½	5	8½	9	23	28
0.013	6¼	7¼	10¼	10	∞	∞
0.017	10¼	11	11¾	11¾		
0.02	12½	13½	17	13¼		
0.023	18	18	17½	19		
0.027	34	39	38	45		
0.03	∞	∞	∞	∞		

[1] The concentration resulting when one volume of the salt solution was mixed with four volumes of plasma. To the salt was added the calculated amount of $CaCl_2$ to have a concentration of 0.005 M in the final mixture.

TABLE 4. INFLUENCE OF VARYING CONCENTRATIONS OF CALCIUM, STRONTIUM AND MAGNESIUM ON THE PROTHROMBIN TIME OF PLASMA DECALCIFIED WITH AMBERLITE

CONCENTRATION OF THE SALT	PROTHROMBIN TIME								
	$CaCl_2$			$SrCl_2$			$MgCl_2$		
	Man	Dog	Rabbit	Man	Dog	Rabbit	Man	Dog	Rabbit
M	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
0.06	18	12	13	∞	∞	∞	∞	∞	∞
0.05	16.5	10.5	11	1620	680	750	∞	∞	∞
0.04	15	8.5	9	36	14.5	14.5	293	174	182
0.03	13.5	8	8.5	28	13.5	13.5	125	81.5	89
0.02	13	7.5	7.5	22	13	13	78	46	43
0.01	12	7.5	7	19.5	12.5	12	64	35.5	31.5
0.005	12	7	7	22	12.5	12	58	32.5	30
0.0025	12	7	6.5	30	18.5	16	69.5	37	38
0.00125	13.5	7	7	37	21	21	74.5	46	45.5
0.0006	15	8	8.5	55	36	33	89	58	57
0.0005	19	8.5	10	83	39	37	∞	∞	∞

plains its marked inhibitory effect on coagulation. By using fixed concentrations of strontium, magnesium and barium but varying strengths of $CaCl_2$ the characteristic wide range of the concentrations of calcium capable of causing an optimum clotting of plasma tends to be more narrow.

The comparative activity of calcium, strontium and magnesium on the prothrombin time. From the results of table 4 one can see that calcium is much more active than strontium but that, surprisingly, even magnesium has a definite even though weak activity. The range of concentrations at which a minimum prothrombin time is obtained is widest for calcium. Significantly, however, a solution as diluted as 0.0006 M $MgCl_2$ will produce coagulation when excess thromboplastin is present. The optimum concentration of strontium and magnesium is essentially the same, but the inhibitory action of magnesium appears to be greater.

TABLE 5. Influence of varying concentrations of strontium, magnesium and barium in presence of a fixed optimum amount of calcium (0.005 M) on the prothrombin time of plasma decalcified with Amberlite

CONCENTRATION OF THE SALT	PROTHROMBIN TIME					
	$SrCl_2$		$MgCl_2$		$BaCl_2$	
	Man	Rabbit	Man	Rabbit	Man	Rabbit
M	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
0.006	12	6.5	12	6.5	12	6.5
0.012	12	6.5	12	6.5	13	7
0.018	12	6	12	6.5	14.5	8.5
0.024	12	6.5	12.5	6.5	17	9.5
0.042	12	6.5	12.5	6.5	19.5	10.5
0.06	15.5	9	14.5	9.5	26.5	15
0.078	25	15	26.5	14	∞	∞
0.102	35	20.5	38	22.5		
0.12	43	28	40.5	26.5		
0.138	114	48	98	42.5		
0.16	165	112	124	79.5		
0.18	302	148	256	124		
0.2	∞	∞	∞	∞		

To test whether this action is real or apparent, the prothrombin time was determined using a fixed optimum concentration of calcium and the same series of dilutions of strontium and magnesium chloride. From the results in table 5 it is obvious that the inhibitory action of strontium and magnesium is practically equal and of the same order of magnitude as the retarding action of calcium itself.

DISCUSSION

It can be concluded that the minimum amount of calcium required for optimum coagulation, as judged by the shortest clotting time, is approximately the same as the concentration normally found in blood. Clotting still occurs, however, at much lower concentrations especially if the amount of thromboplastin present is increased. This possibly explains why Vines (3) found that only one seventeenth of the serum calcium was necessary for coagulation and Stassano and Daumas (4) likewise stated that only 13 to 21 mgm. of calcium per liter of blood were required for clotting. It should be emphasized that the coagulation time as determined ordinarily is influenced mainly by the variations of the available thromboplastin and that the changes in calcium concentration which can occur in physiological or pathological conditions are probably never sufficient to affect the speed of coagulation.

While it is generally accepted that calcium is necessary for the conversion of prothrombin to thrombin, it is still a moot question whether it acts catalytically or stoichiometrically. The former view has been accepted almost unhesitatingly, but the senior author has recently demonstrated (5) that if the concentration of calcium is progressively diminished a point is reached at which the prothrombin conversion is decreased even though excess thromboplastin is present, which clearly suggests a stoichiometric relationship. Our finding that the coagulation begins to be retarded when the calcium concentration is reduced a little below 50 per cent of the normal is likewise in accord with the stoichiometric view.

The possibility that calcium may have a second rôle in coagulation must, in the light of recent developments, be seriously considered. Ferguson (6), in 1934, reported that calcium is necessary for the agglutination and lysis of platelets. Recently the senior author obtained data (7) showing that thromboplastin occurs in the plasma in the form of a precursor which he designated as thromboplastinogen. For the activation of the latter a factor present in platelets is required. A little later, Milstone (8) also recognized that plasma contains a precursor of thromboplastin, which he has named prothrombinokinase. He has reported experiments indicating that calcium is necessary for the conversion of the precursor to active thromboplastin. Since there are findings which indicate that thrombin is necessary for the agglutination of platelets (9) it remains problematic whether calcium functions only in thrombin formation or also in platelets lysis and in the activation of the thromboplastin precursor.

Whether calcium can be replaced by other bivalent ions has been investigated by various authors. It was observed by Arthus and Pagès (10) that strontium had coagulant action, but was less active than calcium. Similar observations were recorded by Heard (11), Ringer and Sainsbury (12) and Mellanby (13). The last three authors claimed that barium chloride also possessed clotting activity, while Arthus and Pagès had found it ineffective. Loomis and Seegers (14) recently found that strontium was a fair but rather slower activator of prothrombin than calcium and that even magnesium and barium had activity, though very weak. Richards and Johnson (15) studied the substitution of strontium for calcium in the one-stage prothrombin method and found that the prothrombin time was much longer and higher concentrations of strontium were required as compared with calcium for optimal activity. The findings of the present investigation confirm the previous observations that strontium has definite but weaker action than calcium in coagulation. Magnesium is ineffective to bring about clotting of decalcified blood and plasma unless a high concentration of thromboplastin is present. It appears, therefore, that the action of calcium in coagulation is not specific, but that strontium and magnesium likewise show this action.

Calcium and also strontium and magnesium exert an inhibitory action on clotting as their concentration is increased above the optimum level. This was observed as early as 1896 by Horne (16) and recently confirmed by Greville and Lehmann (17) who also concluded from their studies that a true antagonism exists between Mg^{++} and Ca^{++} in respect to plasma clotting. Since these ions accelerate or retard clotting purely on the base of their concentration, the coagulation time or prothrombin time is therefore a resultant of a positive or activating and a negative or inhibitory action of calcium and related bivalent ions. Since the activating effect of strontium and magnesium is relatively weak, the inhibitory influence becomes very evident. If the activating action is made constant by selecting, for instance, an optimal concentration of calcium, it is found that the inhibitory effect of calcium, strontium and magnesium is essentially the same and is relatively weak.

It seems logical to assume that the activating effect of calcium, strontium and magnesium takes place directly in the interaction of prothrombin with thromboplastin. The possibility that they may also function in the lysis of platelets must not be ignored. On the other hand, the inhibitory action may perhaps be due to the ability of these ions to stabilize proteins, particularly fibrinogen. It should also be emphasized that extrinsic factors may alter the relative domination of one effect over the other. Thus, in the coagulation of whole blood, the activating power of calcium, strontium and magnesium is comparatively weak due to the limited quantity of thromboplastin available. When excess of thromboplastin is present, then the activating force overshadows the inhibitory and under these conditions magnesium exhibits clotting activity. From the results presented one can find no evidence of a direct antagonism between calcium and the other ions.

SUMMARY

By studying the effect of varying concentrations of calcium chloride added to blood after decalcification by means of Amberlite, it was found that the optimal amount of calcium is approximately the same as normally present in blood. Strontium has a weak clotting activity and a higher molar concentration is required. Magnesium fails to clot decalcified blood.

Calcium, strontium, magnesium and barium have an inhibitory action on coagulation which becomes greater as their concentration is increased above the optimum level. By adding a fixed optimum concentration of calcium and varying amounts of strontium, magnesium and barium to decalcified plasma it was found that the first two have about the same inhibitory action, whereas barium has a much greater anticoagulant effect.

Strontium is decidedly less active in coagulation than calcium in the presence of excess thromboplastin. Magnesium has a weak but definite activity. The molar concentrations required for the shortest prothrombin time are higher for strontium and magnesium than for calcium. With a fixed optimum concentration of calcium the inhibitory effect of strontium and magnesium is found to be approximately the same and of about the same magnitude as that of calcium.

REFERENCES

1. **Steinberg, A.** Proc. Soc. Exp. Biol. & Med. **56:** 124, 1944.
2. **Stefanini, M.** Proc. Soc. Exp. Biol. & Med. **67:** 22, 1948.
3. **Vines, H. W. C.** J. Physiol. **55:** 86, 1921.
4. **Stassano, H. and A. Daumas.** Compt. rend. Acad. Sc. **150:** 937, 1924.
5. **Quick, A. J.** Science **106:** 591, 1947.
6. **Ferguson, J. H.** This Journal **108:** 670, 1934.
7. **Quick, A. J.** Am. J. Med. Sc. **214:** 272, 1947.
8. **Milstone, J. H.** Science **106:** 546, 1947.
9. **Pinniger, J. L. and F. T. G. Prunty.** Brit. J. Exp. Path. **27:** 200, 1946.
10. **Arthus, M. and C. Pagès.** Arch. Physiol. norm. path. **2:** 739, 1890.
11. **Heard, W. N.** J. Physiol. **51:** 294, 1917.
12. **Ringer, S. and H. Sainsbury.** J. Physiol. **11:** 369, 1890.
13. **Mellanby, J.** J. Physiol. **38:** 28, 1909.
14. **Loomis, E. C. and W. H. Seegers.** Arch. Biochem. **5:** 265, 1944.
15. **Richards, R. K. and L. Johnson.** Proc. Soc. Exp. Biol. & Med. **57:** 125, 1944.
16. **Horne, R. M.** J. Physiol. **19:** 356, 1896.
17. **Greville, G. D. and H. Lehmann.** J. Physiol. **103:** 175, 1944.

LIMITATIONS OF THE RENIN-HYPERTENSIN HYPOTHESIS[1]

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The discovery of the action of renin on the serum globulin hypertensinogen and the separation of the polypeptide hypertensin (angiotonin 1, 2) led to the hypothesis that this transformation is enzymatic and that the resulting elevation in blood pressure is a function of newly formed hypertensin. Increased renin activity, it was postulated would result in larger hypertensin production.

Recent studies (3–8) necessitate re-evaluation of the renin-hypertensin theory as an explanation of the humoral mechanism concerned in hypertension. Attempts to demonstrate adequate amounts of renin in the blood of hypertensive patients and animals so far have been unsuccessful. Moreover, it has not been possible to find hypertensin in the blood of hypertensive patients or animals (9) or to detect it in plasma withdrawn at the peak of the pressure curve subsequent to an injection of renin into rabbits (10).

The study that follows supplies further evidence that the blood pressure response to renin cannot be explained adequately by the production of hypertensin.

MATERIALS AND METHODS

Renin preparations free of hypertensinase[2] were made following the method described by Katz and Goldblatt (11) as modified by Dexter (3). Fresh hog kidneys in 5-kgm. batches were prepared in an electric meat grinder and, after the addition of twice their weight of distilled water and thorough mixing, were allowed to stand overnight at 5°C. The material was then strained through five thicknesses of gauze and the pulp discarded. The resulting kidney extract was brought to pH 2.9 ± .05 at 0°C. with 10 per cent trichloracetic acid. Cold saturated NaCl solution was added until the molar concentration was .92 to precipitate the hypertensinase. The 'brei' was then filtered over a period of 5 to 6 hours at +5°C. The resulting clear, yellow filtrate was adjusted to pH 5.0 with 1N NaOH; 400 grams of solid ammonium sulfate per liter of filtrate were added and after 4 or 5 hours it was filtered through large Buchner funnels with E & D filter paper ♯615 and Kieselguhr. The precipitate was redissolved in a small amount of distilled water; it was then dialyzed against distilled water until the dialysate was free of sulfate and the resulting renin solution was lyophilized. This method yielded about three grams of protein per 5-kgm. batch of kidney. Two mgm. were equivalent to one Swingle Dog Unit (12) on direct injection. Hypertensinogen furnished by Dr. Dexter was used in the first experiments. Later, Armour Bovine fraction # IV_1 was secured. In some experiments freshly prepared dog serum freed of hypertensinase was substituted for hypertensinogen.

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[2] Grateful acknowledgement is made to Dr. Dexter for his contribution of hypertensinogen and for confirming that the renin preparation was free of hypertensinase.

Hypertensinogen and renin solutions kept in motion by an electric stirrer were incubated in a water bath at 38°C. Two cc. of 1/15 N phosphate buffer (pH 7.5) were added for every 10 cc. of 1 per cent hypertensinogen solution to maintain a pH of 7.4 to 7.5.

In the experiments with short incubation times, great care was taken to stop the hypertensin formation abruptly. The interruption was accomplished by adding a previously determined amount of 1N HCl to bring the pH to 5.2. The acidified fluid was then immersed in boiling water. Following deproteinization and removal of the precipitate (moist Whatman # 1), the filtrate was lyophilized, the dry product, redissolved in distilled water, was tested for its hypertensin content within the hour by intravenous injection into the foreleg of a nembutalized dog. The femoral artery of such an animal was cannulated and connected with a mercury manometer which recorded the blood pressure on a kymograph. The direct renin assay was carried out in the same way. The technique for perfusion of the rabbit ear has been described (13).

RESULTS

Speed of hypertensin formation. In the course of many experiments it was observed that the average latent period following intravenous injection of renin into the foreleg of a dog is 16 seconds. This accords with values reported in the literature (14). Hypertensin formation obviously must be prompt to be causatively associated with the rise in pressure. The velocity of the catalytic process in vitro is illustrated in the following typical experiments, nos. 1 to 4 (table 1).

As is evident, large amounts of hypertensin were formed after only 20 or 30 seconds incubation of renin with hypertensinogen or serum.

Completeness of transformation of hypertensinogen. Interestingly enough prolongation of the incubation of renin and hypertensinogen to one and one half or three minutes did not increase the hypertensin yield significantly. This is demonstrated in table 1, experiments 5 to 9, and by figures 1, 2, 3 and 4.

The results of the experiments 6, 7, 8 and 9 indicate that 20 to 30 seconds of incubation of 100 cc. of hypertensinogen or serum with 1.5 to 2.5 units of renin were sufficient to result in the total amount of hypertensin the available hypertensinogen is able to yield. The amount of fraction IV_1 (one gram) used in these experiments compares satisfactorily with the possible total quantity of this fraction present in the plasma of a small dog[3].

It can be assumed, therefore, that injection of sufficient renin into the blood stream of a dog rapidly catalyzes the formation of the total possible amount of hypertensin.

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[3] The total plasma proteins of a 5.5 kgm. dog (450 cc. blood, 250 cc. plasma) approximate 14 grams. The α-globulin fraction comprises about one-fourteenth of the total plasma proteins. (E. J. Cohn *et al.*, J. Am. Chem. Soc. **68**: 459, 1946.)

Action of increasing amounts of renin in vitro and in vivo. As has been reported (14) the duration of the blood pressure elevation following hypertensin injection is not more than 2.5 to 3 minutes. In contrast, an injection of only 2 or 3 dog units of renin is followed by a sustained elevation of the blood pressure extending to 20 and sometimes even exceeding 30 minutes. The differences in the two pressor curves are striking (fig. 5). Slow and continuous hypertensin formation has been suggested as the cause of the prolonged elevation of the blood pressure following renin (14). There is no evidence to support this or that there may be delay in the in vivo mixing of the hypertensinogen and renin, two possibilities that should be borne in mind. The observed rapid completion of hypertensin formation in vitro and the absence of information to the contrary in the living animal is noteworthy negative evidence.

TABLE 1

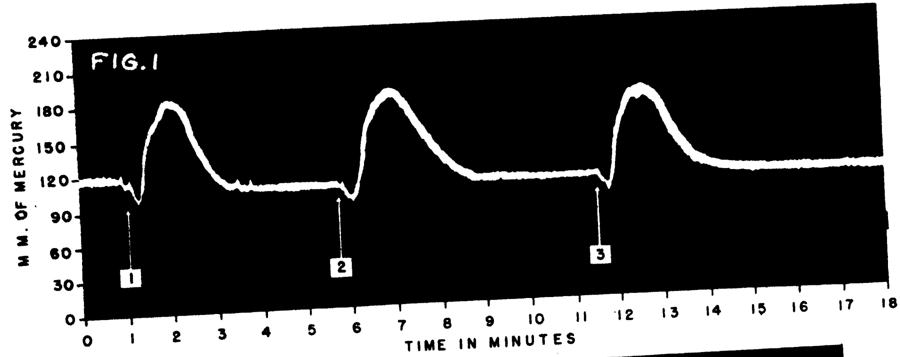
EXPERIMENT	RENIN UNITS	1% HYPERTENSINOGEN (H) OR DOG SERUM (S)	INCUBATION PERIOD	BLOOD PRESSURE RISE	WEIGHT OF DOG
		cc.	*sec.*	*mm. Hg.*	*kgm.*
1[1]	1.5	100 H	30	42	10
2	1.5	100 H	30	71	7.7
3	2.5	100 S	30	86	8
4	2.5	100 S	20	85	7.6
			min.		
5[2]	1.5	100 H	3	58	10
	1.5	100 H	1½	52	
	1.5	100 H	½	42	
6	1.5	100 H	3	73	7.7
	1.5	100 H	1½	74	
	1.5	100 H	½	71	
7	2.5	100 S	3	90	8
	2.5	100 S	½	86	
8	2.5	100 S	1½	70	9
	2.5	100 S	½	74	
9	2.5	100 S	3	85	7.6
	2.5	100 S	⅓	81	
10[3]	2	20 H	5	58	7
	8	20 H	5	54	
11	.5	140 H	10	78	6.8
	1	140 H	10	76	
	2	140 H	10	80	
12	1	100 S	5	68	6.8
	3	100 S	5	74	
	9	100 S	5	78	

[1] Hypertensin yield after 20 to 30 seconds incubation of renin with hypertensinogen or serum (experiments 1 to 4).

[2] Completeness of the hypertensin yield after only 20 to 30 seconds incubation of renin with hypertensinogen or serum (experiments 5 to 9).

[3] Relation of hypertensin yield to quantity of renin (experiments 10 to 12).

On the other hand, the speed and completeness of the in vitro transformation of hypertensinogen probably is an expression of excess renin. This was obtained with 1.5 Swingle units as shown in experiments 5 and 6 and 2.5 units in experiments 7, 8 and 9 (table 1). If these amounts of renin represent excess, increasing quantities should not lead to greater hypertensin yield. That this is the fact was demonstrated in another series of experiments, nos. 10 to 12 (table 1).



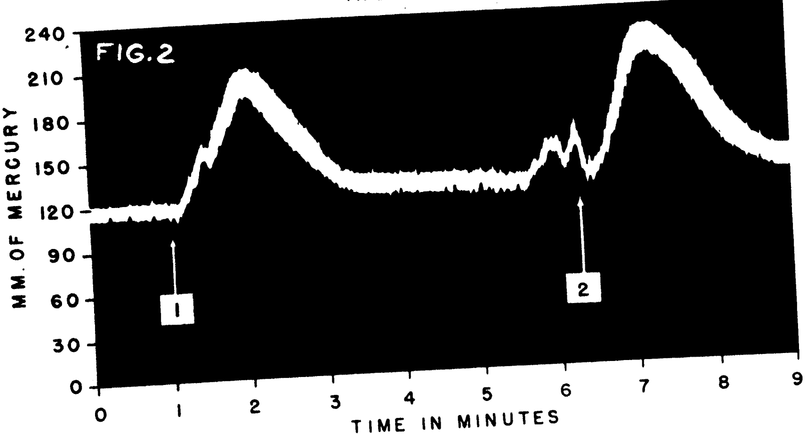
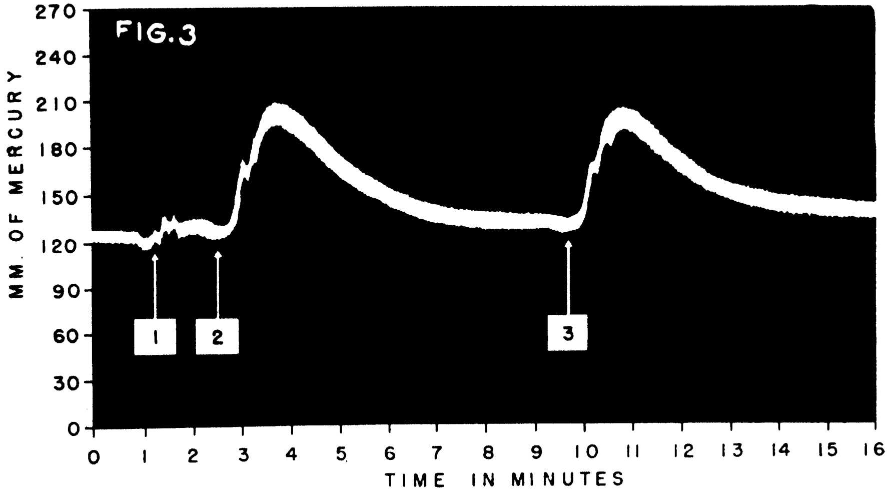


Fig. 1. Blood pressure curves following i.v. injection of hypertensin produced by incubating 1.5 units of renin with 100 cc. of 1 per cent hypertensinogen for: (1) three minutes, (2) one and one-half minutes, (3) one-half minute (Exp. 6, table 1).

Fig. 2. Blood pressure curves following i.v. injection of hypertensin produced by incubating 2.5 units of renin with 100 cc. of dog serum for: (1) one-half minute, (2) three minutes (Exp. 7, table 1).

No significant differences in hypertensin yield were observed with quadrupled renin amounts (experiments 10 and 11). Nine Swingle units of renin yielded almost the same amount of hypertensin as three, and three units only insignificantly more than one (experiment 12, fig. 6). Briefly, if one Swingle unit or multiples thereof are incubated with constant amounts of hypertensinogen, the hypertensin yield is practically the same; this holds even when rather large quantities of hypertensinogen are available. It is difficult to reconcile the unchanged hypertensin yield in in vitro experiments with the well graded increment in blood pressure response to injection of increasing amounts of renin into the living animal. Hessel (15) reported a direct relationship between the quantities



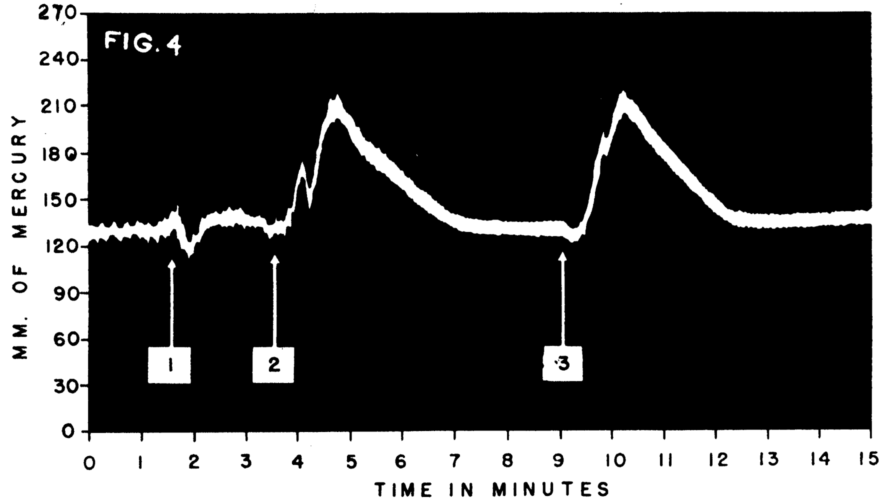


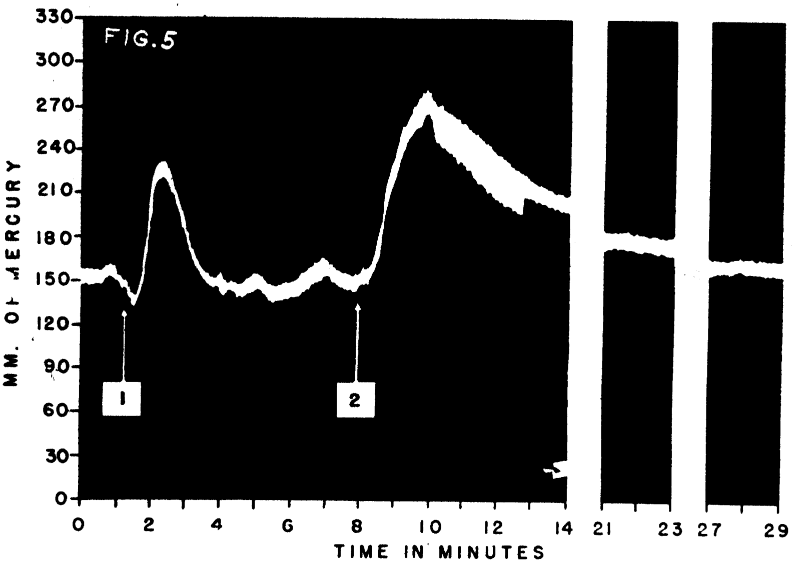
Fig. 3. Blood pressure curves following i.v. injection of hypertensin produced by incubating 2.5 units of renin with 100 cc. of dog serum for: (1) control (no renin added to the serum), (2) one-half minute, (3) one and one-half minutes (Exp. 8, table 1).

Fig. 4. Blood pressure curves following i.v. injections of hypertensin produced by incubating 2.5 units of renin with 100 cc. of dog serum for: (1) control (no renin added to the serum), (2) one-third minute, (3) three minutes (Exp. 9, table 1).

of intravenously injected renin and the blood pressure response, and suggested a blood pressure unit' *E*.

Other investigators reported similar results (16). Swingle and associates (12 defined a renin unit and reported a response of 32 to 52 mm. of Hg for one unit, 55 to 96 for two, 62 to 119 for three and 95 to 132 for four.

Discrepancies are evident between the action of medium amounts of renin (one to 3 Swingle units) in vitro and their pressor action in vivo. Definite differences are also demonstrable when the effects of the injection of large amounts of renin are compared with the effect of large amounts of hypertensin. Pressure rises of 160 to 180 mm. of Hg were often observed following injections of 8 to 10 units of renin (16) and Hessel (15) even reported rises of 250 mm. and more. On the other hand, the maximal blood pressure elevations observed in these studies following injection of hypertensin did not exceed 100 mm. of Hg. These results are in agreement with those of Braun-Menendez and associates. The



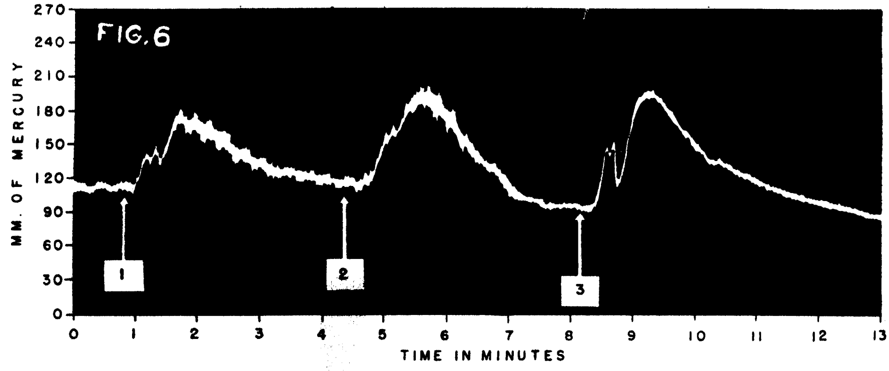


FIG. 5. BLOOD PRESSURE CURVES FOLLOWING: (1) i.v. injection of six units of hypertensin, (2) i.v. injection of four units of renin.

FIG. 6. BLOOD PRESSURE CURVES FOLLOWING I.V. INJECTIONS OF HYPERTENSIN produced by incubating different amounts of renin with constant amounts of hypertensinogen (100 cc. of dog serum). (1) one unit of renin used; (2) three units of renin used; (3) nine units of renin used (Exp. 12, table 1).

latter authors observed maximal elevations of not more than 90 to 100 mm. of Hg, with up to 20 units of hypertensin (14).

Hypertensin yield with increasing quantities of hypertensinogen. The great amounts of hypertensin used in the experiments reported in this paper were prepared with rather large quantities of hypertensinogen. Table 2 shows the relation between hypertensin yield and availability of hypertensinogen.

The dependency of the hypertensin yield on the available hypertensinogen is evident and has been reported previously (14). The influence of the quantity of renin used in this series is insignificant, as can be seen from the similar hypertensin yield.

The reason for the different action of renin in vivo is obscure. Renin still is a crude mixture containing many enzymes. The possibility arises that it may contain a substance which acts directly on effector cells and that this second effect may be demonstrable. An effort to accomplish this involved amputation and perfusion of one ear of a rabbit, intravenous injection of renin or hypertensin followed by removal and perfusion of the second ear.

TABLE 2. RELATION OF HYPERTENSIN YIELD TO THE AVAILABLE HYPERTENSINOGEN

EXPERIMENT	RENIN UNITS 'SWINGLE'	1% HYPERTENSINOGEN	FRACTION OF THE TOTAL HYPERTENSIN YIELD INJECTED	BLOOD PRESSURE RISE	WEIGHT OF DOG
		cc.		*mm. Hg*	*kgm.*
13	4	40	Total	57	7
	4	80	One half	63	
	4	320	One fourth	66	
14	1	20	Total	42	
	1	80	One half	65	7.2
	1	160	One half	90	
15	2	20	Total	30	
	2	100	One half	30	5.4
	2	150	One fourth	40	
	2	310	One sixth	50	

Preliminary study showed that the initial perfusion rates of both ears of normal rabbits did not vary significantly when the ears were amputated within 30 minutes of each other. The variations with 10 pairs of ears were only 10 to 20 per cent of the drop rate.

The effect of intravenous injection of renin. One ear of a normal rabbit was amputated and the basal drop rate determined. Two to three 'Swingle' dog units of renin were then injected into the animal intravenously. Five to fifteen minutes later the second ear was amputated and perfused with 20 cc. of Ringer-Locke to insure absence of blood. The initial drop rate of the second ear in each of 12 perfusion experiments with Ringer-Locke was 40 per cent to 98 per cent less than that of the control ear and remained at this rate for at least five minutes. On continuation of the perfusion with Ringer-Locke it gradually returned over a period of 10 to 30 minutes toward the basal rate of the control ear.

The effect of intravenous hypertensin injection. The identical experiments were carried out in six rabbits using intravenous hypertensin injections instead of renin, with markedly contrasting results.

TABLE 3. Renin influences the vessels of the rabbit's ear in a manner different from that of hypertensin

EXPERIMENT	EAR	DROP RATE PER ½ MIN.	RENIN UNITS 'SWINGLE'	TIME IN MINUTES FROM INJECTION TO AMPUTATION OF EAR
16	First	28	—	—
	Second	4	3.0	5
17	First	38	—	—
	Second	1	2.5	5
18	First	28	—	—
	Second	5	3.0	5
19	First	26	—	—
	Second	10	2.0	5
20	First	44	—	—
	Second	5	3.0	6
21	First	38	—	—
	Second	14	2.0	5
			HYPERTENSIN UNITS	
22	First	23	—	—
	Second	53	3.0	1.5
23	First	24	—	—
	Second	30	3.0	1.25
24	First	34	—	—
	Second	50	3.0	1.0
25	First	18	—	—
	Second	30	6.0	0.75
26	First	20	—	—
	Second	55	6.0	0.75
27	First	30	—	—
	Second	46	7.0	0.75

After the basal drop rate of the first ear had been determined, 3 to 7 units of hypertensin were injected into a rabbit. At the peak of the pressor response (between 45 and 90 seconds after the injection) the second ear was removed, flushed out with 20 cc. of Ringer-Locke and the drop rate measured within three minutes of the time of the hypertensin injection. In no instance was there any reduction in drop rate; there was rather a prompt increase, indicating vasodilation, which subsided after a minute or two to the basal level of the first ear. Table 3 contains the data of typical experiments.

SUMMARY AND DISCUSSION

Amounts of renin just detectable by bioassay in a dog are capable of rapidly and completely transforming large quantities of hypertensinogen. An increase in renin over and above these amounts does not augment the hypertensin yield significantly, the latter depending exclusively upon the available amounts of hypertensinogen. No evidence is at hand to indicate that the quantities of hypertensinogen in the blood can increase consequent to injection of renin. It may be concluded, therefore, that the proportional increase in blood pressure that follows the intravenous injection of increasing amounts of renin cannot be explained by a greater formation of hypertensin.

Differences between the renin and hypertensin effect on blood vessels have been demonstrated: A constrictor effect of intravenously injected renin on the vessel walls of the rabbit's ear is observable after the organ has been removed from the body and perfused with Ringer-Locke for many minutes. No such effect can be demonstrated following an injection of hypertensin.

The possibility is suggested that an unknown factor contained in renin may either prolong or intensify the action of hypertensin or may act independently of it. Recent reports are of interest in this connection. Tripp (17) suggests that hypertensin (angiotonin) lacks some pressor material resulting from the renin and substrate reaction. Helmer and Shipley (18) believe they have evidence of the presence of a pressor substance distinct from renin and originating in the kidney.

Results included in this communication permit the conclusion that the in vivo renin effect is not adequately explained by hypertensin formation.

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REFERENCES

1. Braun-Menendez, E., J. C. Fasciolo, L. F. Leloir and M. J. Munoz. J. Physiol., **98**: 283, 1940.
2. Page, I. H. and O. M. Helmer. J. Exp. Med. **71**: 29, 1940.
3. Dexter, L., F. W. Haynes and C. Bridges. J. Clin. Invest. **24**: 62, 1945.
4. Dexter, L. and F. W. Haynes. J. Clin. Invest. **21**: 627, 1942.
5. Taquini, C. and J. C. Fasciolo. Am. Heart J. **32**: 357, 1946.
6. Haynes, F. W., L. Dexter and R. E. Seibel. This Journal **150**: 198, 1947.
7. Haynes, F. W., and L. Dexter. This Journal **150**: 190, 1947.
8. Grollman, A. Recent Progress in Hormone Research, New York: Academic Press, Inc. **1**: 371, 1947.
9. Gregory, R., P. L. Ewing, W. L. Levin and G. T. Ross. Arch. Int. Med. **76**: 11, 1945.
10. Landis, E. M., E. D. Thomas and J. E. Wood III. Federation Proc. **4**: 43, 1945.
11. Katz, Y. J. and H. Goldblatt. J. Exp. Med. **78**: 67, 1945.

12. Swingle, W. W., H. R. Taylor, W. D. Collings and W. H. Hays. This Journal **127**: 768, 1939.
13. Mylon, E., F. H. Horton and R. P. Levy. Proc. Soc. Exp. Biol. Med. **66**: 1947.
14. Braun-Menendez, E., J. C. Fasciolo, L. F. Leloir, J. M. Munoz, H. C. Taquini. Renal Hypertension. Springfield, Ill. Charles C Thomas: pp. 118, 147, 96, 103, 1946.
15. Hessel, G. Klin. Woch. **24**: 843, 1938.
16. Winternitz, M. D., E. Mylon, L. L. Waters and R. Katzenstein. Yale J. Biol. Med. **12**: 623, 1940.
17. Tripp, E. Federation Proc. **6**(1): March, 1947.
18. Helmer, O. M. and R. E. Shipley. This Journal **150**: 353, 1947.

EFFECT OF INTRAVENOUS INJECTION OF MAGNESIUM CHLORIDE ON THE BODY TEMPERATURE OF THE UNANESTHETIZED DOG, WITH SOME OBSERVATIONS ON MAGNESIUM LEVELS AND BODY TEMPERATURE IN MAN

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The mean serum magnesium concentration in normal persons has been reported variously as from 2.00 to 2.74 mgm. per cent, with a range from 1.60 to 3.66 mgm. per cent (5). Haury and Cantarow (5) found in 52 subjects normal limits from 1.7 to 3.1, with a mean of 2.48 mgm. per cent. Higher concentrations produce well known pharmacological actions (reviewed by Smith, Winkler and Hoff, 12). Vasodilation occurs with concentrations as low as 2 to 5 mEq. per liter (1 mEq. equals 1.2 mgm. per cent), and this is, according to Haury (4), a peripheral action, and according to Winkler *et al.* (17) an action upon the arterioles. Greater concentrations affect the nervous system, first with a 'curare-like' action of retarding neuromuscular transmission (7, 16). With serum concentrations of 6 to 14 mEq. per liter muscles fail to respond to single shocks through the nerve, and with higher concentrations the response to first low and then high frequency stimulation disappears. Still higher concentrations cause depression of the central nervous system.

The temperature-depressing effects of magnesium are less well known, though described in 1916 by Schütz (11) in rabbits. He thought the action was on the temperature-regulating centre, but this is not certain from his work in view of the known peripheral actions of the drug. A number of papers from the laboratory of H. G. Barbour (17, 18) also fail to give conclusive evidence as to the site of action and mechanism. Our interest was renewed on reading that Suomalainen (13, 14) had found an elevated ratio of magnesium to calcium in the serum of hibernating hedgehogs. A single experiment by one of us in 1937 on an unanaesthetised cat gave the result that magnesium injection caused panting at a lower body temperature than usual and suppressed shivering on cooling.

Since magnesium, unlike most antipyretics, is a simple inorganic ion and is somehow concerned in hibernation, a specific effect on the temperature-regulating centre, if it could be demonstrated, would be of considerable interest and might even suggest that the level of serum magnesium was concerned in the normal regulation of body temperature.

Since the centre is extremely sensitive to anaesthetics (6), studies on the normal regulation must be made on unanaesthetised animals, and dogs were chosen for this reason. The plan was to study the mechanism of lowering of body temperature after injection of magnesium in three sets of conditions; *a*) at ordinary room temperature, where there was initially neither shivering nor panting; *b*) while the animal was being warmed and panting moderately; and *c*) while the animal was cooled and shivering moderately. In each case the particular regulating mechanism involved could show increase, decrease or no change.

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METHOD

For each experiment the dog was placed on the animal table and wrapped in a special rubber 'blanket' through which warm or cold water could be circulated under controlled conditions to warm or cool the animal (fig. 1). The deep rectal temperature and the surface temperature of the large pad of the right forepaw were taken by means of thermocouples. The paw temperature served as an indication of vasoconstriction or vasodilation. When the rectal and paw temperatures had become stabilized molar magnesium chloride in various amounts was injected into a saphenous vein and the effects observed.

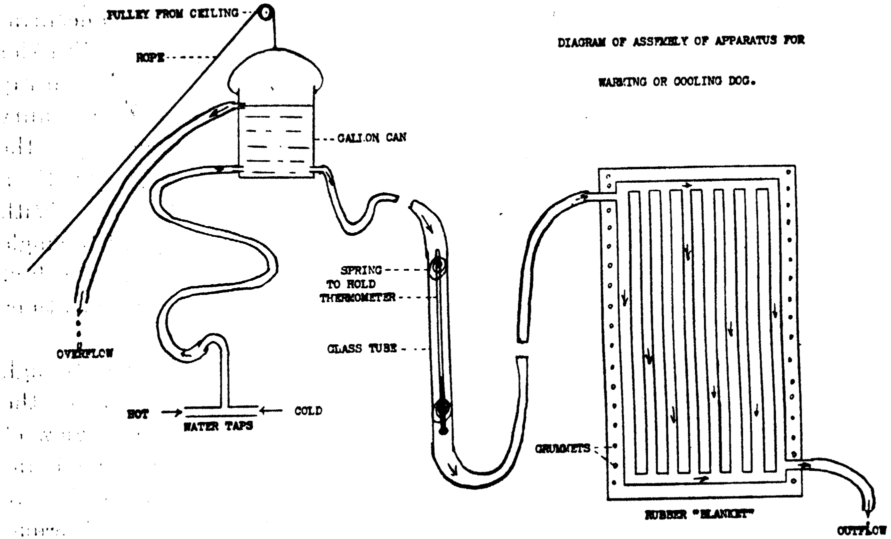

FIG. 1. DIAGRAM OF ASSEMBLY OF APPARATUS for warming or cooling dog.

Samples of venous blood were withdrawn during the experiments and the serum magnesium concentrations were estimated either by a modification of Hoffman's method (8) using 8-hydroxyquinoline, or, in later experiments, by a modification of Garner's method (3) using Titan yellow.

The degree of shivering or of panting, when present, was estimated by inspection, and for comparative purposes was evaluated as 1, 2, 3 or 4 plus. In the warming experiments the apparatus was adjusted so that before the injection the dog maintained a stable rectal temperature by moderate intermittent panting, and this was recorded as two plus panting. In cooling experiments the temperature of the cooling water (about 7°C) was such that initially the dog maintained a stable rectal temperature by means of vasoconstriction and moderate shivering. This is recorded as two plus shivering.

OBSERVATIONS

General effects of injection of magnesium. Usually the dog had been quite active before entering the apparatus and was warm and panting at the beginning of the experiment. The animal became quiet in the apparatus and the paw and rectal temperatures became stabilized. When magnesium was injected intravenously the effect varied with the dosage, from mild or moderate depression to complete paralysis or even narcosis. In all the experiments the dog salivated profusely during and immediately following the injection. In almost all the experiments there was a short period of retching after the injection. The dog usually became drowsy during part of the time after the injection, and there was depression of the Achilles' tendon reflex, which was from moderate to complete, depending on the dose.

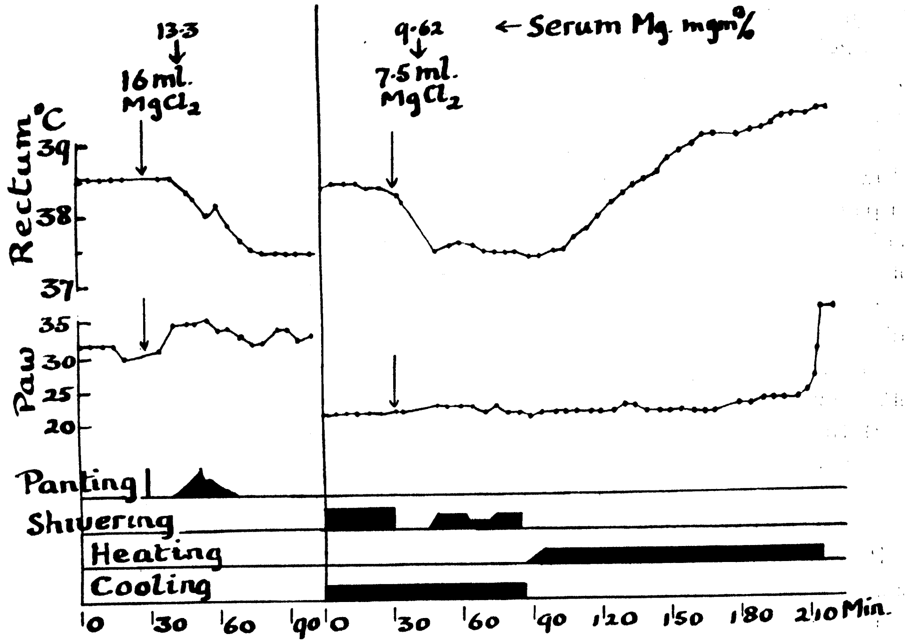

FIG. 2. CHARTS OF TWO EXPERIMENTS. Left: 20-kgm. dog at room temperature. Right: 10-kgm. dog first cooled, then warmed. Molar magnesium chloride was injected intravenously at the point shown by the arrows.

Effects on temperature regulation. Figure 2 shows two experiments, the first at room temperature and the second with cooling followed by warming, to illustrate the results. The drop of rectal temperature after injection of magnesium in each case indicates the antipyretic action, but the mechanism is obviously different. In the first experiment, the rise of paw temperature indicates a vasodilation and there was also stimulation of panting. In the second case, during cooling, shivering was inhibited, while there was no evidence of panting or vasodilation, even though the latter occurred reflexly after warming (at time 210 minutes). The results of 20 experiments on three dogs are summarized in table 1, where only the maximal changes of rectal and paw temperatures are given. The mean values at the foot of the tables should of course be regarded with suspicion, as the data is heterogeneous, but they serve to summarize the findings.

When panting was present it was typical polypneic panting with the mouth held open, rapid rhythmic shallow respiration, and rhythmic movements of the tongue and labial commissures synchronous with respiration. When the dose of magnesium was high enough to produce symptoms of paralysis at the same time that panting was stimulated, there was a striking contrast between the efforts of the animal to pant and the general depression.

Injection of magnesium was always followed by a fall of rectal temperature. However, the changes occurring in peripheral blood flow (as shown by changes in paw temperature), in panting or in shivering when present, differed according to the condition of the animal with regard to temperature regulation at the time of the injection. From table 1 the effects can be summarized as follows:

a) In an animal being warmed there was an increase in panting, and a vasodilation in the cases where this was not already maximal.

b) In an animal neither warmed nor cooled there was vasodilation always, often accompanied by panting.

c) In an animal being cooled there was a decrease or cessation of shivering. Vasoconstriction, however, persisted.

DISCUSSION

Possible mechanisms by which these changes in temperature regulating functions could be produced are;

a) Mechanisms independent of the temperature-regulating centre, such as 1. peripheral vasodilation 2. paralysis of the neuromuscular junction by a curare-like action of magnesium and 3. a depression of metabolism.

b) Mechanisms involving the centre, such as 1. depression of temperature regulation against cold, either specific or part of a general depression of the central nervous system, and 2. stimulation of the central regulation against heat.

The one feature that is common to all the experiments, in the different conditions, is a fall of rectal temperature after the injection of magnesium chloride. Since control injection of sodium chloride did not affect body temperature (and this is well known), it is concluded that the effect is due to the magnesium ion.

Because of the pharmacological actions of magnesium it is necessary to consider certain possibilities whereby the body temperature might be affected independently of the temperature-regulating centres. Magnesium is a powerful vasodilator, and this action is reputed to be peripheral (4, 17). Also, magnesium exerts a powerful paralytic action at the neuromuscular junction—its so-called 'curare-like' action (7, 16). Either of these actions could influence temperature

TABLE 1.—SUMMARY OF EXPERIMENTS

WT. IN KILOS	ROOM TEMP.	M. $MgCl_2$ INJECTED	CHANGE IN RECTAL TEMP. AFTER Mg	PAW TEMP.		PANTING		REMARKS
				Initial temp.	Change on injtn.	Before	After	
A. Dog neither warmed nor cooled								
	°C.	ml.	°C.					
9.6	21	4.8	−0.40	Stayed warm		0	++++	Rectal temp. still falling when expt. ended. Paw temp. estimated by palpation only.
20	24	16.0	−1.04	29.7	+5.4	0	++++	Panted during injtn., evidence of narcosis by Mg. Increased panting when narcosis wore off.
20	26	5.0	−0.35	34.2	+0.9	0	+++	Serum Mg 4.23 mg. % 25 mins. after injection.
10	25	5.0	−0.35	34.4	+1.2	0	++	Serum Mg 9.04 mg. % 22 mins. after injection.
10	22	7.5	−0.98	22.4	+9.6	0	0	Serum Mg 10.04 mgm. % 7 mins. after injection.
10	24	10.0	−0.95	22.5	+4.3	0	++++	Serum Mg 10.9 mgm. % 9 mins. after injection.
10	26	9.5	−0.49	33.0	+1.4	Occas. panting	+++	Serum Mg 2.04 mgm. % before, 7.60 mgm. % 12 mins. after injection.
Mean values...	24		−0.65	29.4	+3.8	0	+++	
B. Dog warmed								
9.6	27	4.8	−0.87	36.7	+1.5	++	++++	
20	21	5.0	0	36.1	−3.8	++	++++	First expt. on this dog. Struggle during injtn. raised rectal temp. Constriction may be psychogenic.
9.6	26	9.6	−1.43	36.0	+0	++	++++	Curare-like action of Mg obvious. Panting very severe.
20	20	6.0	−0.56	25.5	+9.0	++	++	

TABLE 1—Continued

WT. IN KILOS	ROOM TEMP.	M. $MgCl_2$ INJECTED	CHANGE IN RECTAL TEMP. AFTER Mg	PAW TEMP.		PANTING		REMARKS
				Initial temp.	Change on injtn.	Before	After	
	°C.	ml.	°C.					
10	27	5.0	−0.25	35.3	+1.2	+	++++	Serum Mg 2.33 mgm. % before, 7.12 mgm. % 16 mins. after injtn.
10	27	7.5	−0.25	34.9	+1.2	0	++	Serum Mg 9.87 mgm. % 12 mins. after injection.
10	24	7.5	−0.32	33.5	+0.5	++	++++	Serum Mg 2.23 mgm. % before, 7.59 mgm. % 15 mins. after injection.
Mean values...	25		−0.61	33.7	+2.2	+or++	++++	2nd expt. omitted from mean.
C. Dog cooled								
						SHIVERING		
						Before	After	
9.6	16	4.8	−0.50	17.5	−1.4	++++	++	
20	19	1.0	−0.04	23.3	−0.3	+++	++	Some struggling on injection.
20	23	10.0	−0.88	26.3	+2.7	++	0	Panting ++++ immediately on injection. Serum Mg 6.0 mgm. % 30 mins. after injection.
10	21	5.0	−1.05	22.0	+1.0	++	0	Rectal temp. still falling at end of expt. Serum Mg 2.73 mgm. % before, 6.44 mgm. % 10 mins. after injection.
10	23	7.5	−0.90	21.4	+1.1	+++	0	Serum Mg 9.62 mgm. % 15 mins. after injection.
20	20	15.0	−0.81	21.1	+3.2	++	0	
Mean values..	20		−0.70	21.9	+1.0	+++	+	

without directly affecting the temperature-regulating centres. Increased concentrations of magnesium cause central depression and narcosis, and it is possible that general depression would also depress temperature regulation. The problem is to distinguish any action on the temperature-regulating centres from these other nonspecific actions of the ion.

Vasodilator action. In the warming experiments and most experiments at room temperature, marked vasodilation occurred if it was not present initially. This is in accord with the accepted vasodilator action of magnesium. However, in the cooling experiments vasoconstriction was maintained despite the reported peripheral vasodilator action of magnesium. It appears, therefore, that the vasoconstrictor centre is not significantly depressed by magnesium in the amounts used, for it overrides the tendency of magnesium to cause peripheral vasodilation.

Action on shivering—neuromuscular paralysis or central depression? In the cooling experiments magnesium consistently caused a decrease or cessation of shivering without marked evidence of vasodilation. The effect on shivering could be due to depression of the centre responsible for shivering. If so, the depression might be part of general depression of the central nervous system, or it might be specific on the temperature-regulating centre. In some experiments the dog showed gross paralysis, and in these at least there is no doubt that a curare-like effect of the ion could be adequate to explain the effect on shivering. In other experiments gross paralysis did not occur, but there were usually signs of depression, and in general there was a positive correlation between the fall in rectal temperature and the evidence of general depression. This is in agreement with Schütz (11), who noted a relation between the temperature depression and the symptoms of paralysis after administration of magnesium to rabbits. It is possible, therefore, that the curare-like action of magnesium could account for the effects on shivering. There may be also a central effect that influenced shivering, but it was not distinguished in these experiments from general depression of the central nervous system.

TABLE 2.—MEAN SERUM MAGNESIUM CONCENTRATIONS OF SUBJECTS ARRANGED ACCORDING TO ORAL TEMPERATURE

ORAL TEMPERATURE	MEAN SERUM MAGNESIUM CONCENTRATION MGM. PER 100 CC.	
	Healthy subjects	Patients
°*F.*		
97.0–97.4	2.25 (7)[1]	
97.5–97.9	2.28 (10)	2.29 (2)
98.0–98.4	2.22 (21)	2.29 (7)
98.5–98.9	2.36 (7)	2.25 (7)
99.0–99.4		2.29 (2)
99.5–99.9		2.23 (1)

[1] Figures in brackets show number of observations.

Action on panting—stimulation or depression? We have seen that temperature depression by means of vasodilation, and by means of decrease or cessation of shivering, might be explained on the basis of accepted peripheral actions of the magnesium ion. The effect on panting, however, can not be explained by known peripheral actions of the ion. By its nature, panting must be a central effect.

Superficially, it would appear to represent stimulation of regulation against heat. But Lilienthal and Otenasek (9) have described a panting centre that is separate from the hypothalamic centres that regulate body temperature, and they point out the possibility of release phenomena affecting panting. It is possible, therefore, that magnesium may cause panting by releasing such a centre from controlling influences, and if this were so it would be consistent with the general depressing action of magnesium (10). However, against this is the observation that during general depression by means of nembutal, used in one warming experiment, panting stopped and body temperature rose. In any case, panting is increased by magnesium injection, and any argument as to 'direct' stimulation of a centre or 'release' from the influence of other centres by their depression is largely academic, especially in view of the well-known reciprocal actions in the central nervous system.

One possible mechanism mentioned for the effect of magnesium on body temperature is depression of metabolism. In the present series of experiments no attempt has been made to investigate an effect of magnesium on resting metabolism, because the obvious effects of magnesium—panting, decrease in shivering and increase in paw temperature—are of such importance to temperature regulation that the first problem is to determine whether they represent a true antipyretic effect, mediated through the temperature-regulating centres. Berta and Györi (2) found that subcutaneous injection of magnesium gluconate lowered the oxygen consumption of the rat 20 to 40%. It might be supposed that this effect was secondary to neuromuscular paralysis by magnesium, but these authors point out that Tangl and Verzar (15) found that in animals that were accustomed to metabolism experiments neuromuscular paralysis by curare did not lower the metabolic rate.

CORRELATION OF SERUM MAGNESIUM LEVELS AND BODY TEMPERATURE IN MAN

To investigate the possibility that magnesium may play a physiological role in determining the normal body temperature, clinical observations were made of the serum magnesium concentration in relation to the body temperature at various times of day. Sets of two or more observations in a 24-hour period were made on 10 normal subjects and six ambulatory patients. Forty-five determinations on 10 healthy subjects gave a mean value of 2.26 ± 0.33 SD mgm. per cent and 19 on ambulatory patients gave 2.26 ± 0.15 SD, the total 64 determinations yielding a mean of 2.26 ± 0.30 SD. The results are listed in relation to the levels of oral temperature in table 2, and it is apparent that there is no clear correlation. This was further tested by comparing the magnesium levels for each subject when the lowest oral temperature and the highest oral temperature were recorded. The results are shown in table 3. Again, it is seen that there is no constant trend.

An additional series of observations was made on six patients receiving artificial fever therapy in the fever cabinet and on one patient who had artificial fever induced by typhoid vaccine. In the patients in the fever cabinet the mean serum magnesium concentration was 2.32 ± 0.30 SD mgm. per cent before fever when the mean temperature was 99.2°F, and 2.19 ± 0.26 SD mgm. per cent at the height of fever when the mean oral temperature was 105.2°F. The patient who received vaccine did not develop much fever, and successive temperature and magnesium figures were 98.6° and 2.45 mgm. per cent; 99.6° and 2.40 mgm. per cent; and 100.6° and 2.39 mgm. per cent. It is apparent that a large number of cases would be needed to prove a significant relation, but in any event the change in magnesium concentration with change of body temperature, in the physiological range, is too small to lead to significant physiological effects.

SUMMARY

1. On intravenous injection into the dog magnesium chloride causes a decrease in rectal temperature whether the dog is in an environment that is warm, cool or at normal room temperature.

TABLE 3.—COMPARISON OF MEAN SERUM MAGNESIUM CONCENTRATIONS WHEN THE LOWEST ORAL TEMPERATURES WERE RECORDED WITH THOSE WHEN THE HIGHEST ORAL TEMPERATURES WERE RECORDED

	MEAN TEMPERATURE		MEAN SERUM MAGNESIUM CONCENTRATION
		°F.	*mgm. per 100 cc.*
18 sets of observations on 10 normal subjects	Low	97.7	2.16
	High	98.3	2.32
6 sets of observations on 6 patients	Low	98.2	2.38
	High	98.9	2.23
24 sets of observations on 16 subjects (Total)	Low	97.8	2.22
	High	98.4	2.29

2. In a cold environment the effect is produced by a decrease of shivering and of voluntary motor activity without vasodilation.
3. In a comfortable environment the effect is produced either by panting or by vasodilation or a combination of these, and probably by a decrease in muscular tone in some cases.
4. In a warm environment the effect is produced by an increase in panting.
5. It is probable that the decrease in shivering is due to the curare-like action of magnesium on the neuromuscular junction. If there is depression of the temperature-regulating centre, it has not been distinguished from the general depression of the nervous system.
6. The experimental results are not inconsistent with the view that the vasodilator action of magnesium is a peripheral action. However, in the shivering animal the vasoconstrictor centre overrides the vasodilator action of magnesium.
7. The increase in panting must result from a central effect of magnesium. While this appears to represent stimulation of the panting centre, it may of course be due to a 'release phenomenon' when other 'inhibitory' centres are depressed.
8. There is no clear evidence that magnesium exerts an antipyretic action spe-

cifically through the temperature-regulating centres. However, its combined actions change the level of *effective* regulation in favour of a lower body temperature rather than merely depressing the temperature-regulating mechanism.

9. In a short series of investigations on healthy human subjects and ambulatory patients no evidence was found that there is a significant relation between the diurnal fluctuation of normal body temperature and the serum magnesium concentration. Neither was a significant relation found between the serum magnesium concentration and the body temperature during artificial fever. The changes in magnesium level with body temperature are too small to be of physiological importance.

We wish to express our thanks to Mrs. Martha McIntosh for her able assistance in conducting the experiments reported in this paper.

REFERENCES

1. Barbour, H. G. and J. E. Winter. Proc. Soc. Exp. Biol. & Med. **25:** 582, 1927.
2. Berta, L. and G. Györi. Biochem. Z. **311:** 81, 1942.
3. Garner, R. J. Biochem. J. **40:** 828, 1946.
4. Haury, V. G. J. Pharmacol. Exp. Therap. **65:** 453, 1939.
5. Haury, V. G. and A. Cantarow. J. Lab. Clin. Med. **27:** 616, 1941.
6. Hemingway, A. This Journal **134:** 350, 1941.
7. Hoff, H. E., P. K. Smith and A. W. Winkler. This Journal **130:** 292, 1940.
8. Hoffman, W. S. Photelometric Clinical Chemistry. New York: Morrow, 1941.
9. Lilienthal, J. L. Jr. and F. T. Otenasek. Bull. Johns Hopkins Hosp. **61:** 101, 1937.
10. Meltzer, S. J. and J. Auer. This Journal **21:** 400, 1908.
11. Schütz, J. S. Arch. exp. Path. Pharmakol. **79:** 285, 1916.
12. Smith, P. K., A. W. Winkler and H. E. Hoff. Anesthesiology **3:** 323, 1942.
13. Suomalainen, P. Nature, London. **141:** 471, 1938.
14. Suomalainen, P. Annals. Acad. Scientarium Fennicae Ser. A. v. **53:** No. 7: 1, 1939.
15. Tangl, F. and F. Verzar. Biochem. Z. **92:** 318, 1918.
16. Winkler, A. W., H. E. Hoff and P. K. Smith. Yale J. Biol. & Med. **13:** 123, 1940.
17. Winkler, A. W., P. K. Smith and H. E. Hoff. J. Clin. Invest. **21:** 207, 1942.
18. Winter, J. E., C. H. Richey and H. G. Barbour. J. Pharmacol. & Exp. Therap. **38:** 343, 1930.

EFFECT OF PULMONARY ARTERY LIGATION ON THE HISTAMINE CONTENT OF LUNG, WITH OBSERVATIONS ON CONCOMITANT STRUCTURAL CHANGES[1]

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The high concentration of histamine in the lung, and in certain species the liver, suggests that those organs which first receive the venous effluent from the various tissues may function to remove histamine from the systemic circulation and store it in an inactive form (1). To test this hypothesis the effect of pulmonary artery ligation over varying periods of time on the histamine content of the dog lung was determined. It was reasoned that if pulmonary tissue functions to filter and store histamine, marked differences in histamine concentration between ligated and control lobes would become apparent over a period of time.

Early experiments were of short duration in order to determine whether or not ligation of the pulmonary artery would be compatible with the maintenance of structural integrity. The ability of the bronchial circulation to maintain the pulmonary parenchyma, a finding which will be discussed in more detail in a later paper, permitted long range observations which afforded a definitive answer to the effect of pulmonary artery ligation on the histamine content of lung tissue.

METHODS

Ten stock dogs weighing around 10 to 15 kilograms were used. Anesthesia was induced with pentobarbital and supplemented when necessary by the intratracheal insufflation of ether-air mixtures through a soft rubber catheter. Under aseptic technic, an intercostal incision was made in the right chest. The right middle lobe was removed by individual dissection and ligation of pulmonary artery, vein and bronchus in that order and preserved for histamine assay. The pulmonary artery branch to the lower lobe was then freed and ligated doubly with silk in continuity. The remaining lobes were protected against direct trauma during the course of the operation and carefully reinflated to fill the pleural cavity during the closure. There were no operative deaths during the series.

At intervals ranging from two weeks to eight months, the animals were killed by overdosage with intravenous pentobarbital. At autopsy the condition of the remaining lobes in situ was noted. The occlusion of the pulmonary artery to right lower lobe was verified. The right and left lungs were removed separately. In the later animals of the series the weight and collapsed volume of the right and left lower lobes were recorded. The bronchi of the two lobes were then cannulated, and the two lobes brought simultaneously to inflation by air injection

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[1] Aided by a Grant from the Fluid Research Funds, Yale University School of Medicine.

under control with a water manometer. The cannulae were then occluded and the expanded volume of each lobe measured by water displacement; the average of three determinations was recorded. In all experiments representative specimens from the peripheral and hilar portions of the middle lobe (excised at operation), and the lower lobes (removed at autopsy) were weighed and placed in a refrigerator until treated within a few hours according to the procedure outline under assay technic. Additional specimens were immersed in ten per cent formalin and prepared for histologic study by hematoxylin-eosin and Masson connective tissue stains.

Assay technic. Tissues were kept in the refrigerator from the time they were obtained until extracted, usually a period of no more than six to eight hours. Control experiments showed that pulmonary tissue may be kept at icebox temperature for 48 hours without any change in histamine content (2).

From one to three grams of tissue was extracted for histamine assay. The samples were thoroughly macerated by grinding with sand, the tissue pulp and sand were transferred to a wide-mouthed bottle and extracted for three hours in a mechanical shaker with 50 cc. of 70 per cent alcohol containing sufficient HCl to make a 0.1 N solution. The supernatant fluid was decanted, the residuum re-extracted, and the combined extracts were centrifuged. The resultant supernatant fluid was evaporated to dryness on a steam bath. The extract was kept in the form of this dried residue until the time of bioassay. Immediately prior to bioassay, the dried residue was taken up in an amount of Tyrode's solution such that the extractives of each gram of the original lung sample were dissolved in 5 cc. The resultant solution, free of sediment, was employed in the bioassay and either used as such or further diluted with Tyrode's solution.

Assay was performed on isolated segments of guinea pig ileum suspended in 3 cc. of oxygenated Tyrode's solution containing one mgm./liter of atropine sulfate. Samples from the previously resected right middle lobe, the right lower lobe and the left lower lobe were assayed on the same strip of ileum using crystalline histamine as the standard.

The technic of extraction described above fulfills the known requirements for the quantitative extraction of histamine from tissue and is simple in execution. Seventy per cent acid alcohol not only supplies a medium in which histamine is readily soluble but also acts as a bactericidal agent and a protein precipitant. Thus the formation of histamine by bacterial action is circumvented and the bound cellular histamine is released. Control experiments have shown that 100 per cent of known amounts of histamine added to tissue can be recovered by this procedure.

RESULTS

The results of these experiments are presented in two categories: first, the effect of excluding the pulmonary arterial flow on the histamine content of the right lower lobe as compared with the content in the preoperative (R.M.L.) and postoperative (L.L.L.) controls; secondly, the observed effect of pulmonary artery ligation on the structural integrity of the lobe. While the latter was not a primary purpose of the experiment, the results are of considerable interest and appear worthy of recording.

The data of table 1 show that with a single exception the long-standing exclusion of mixed venous (pulmonary artery) blood from a single lobe for a period ranging from 15 to 256 days had no appreciable effect on the tissue histamine level as determined by the bioassay method.

TABLE 1. Histamine levels in pulmonary tissue
(Micrograms per gram, wet weight)

	DOG NUMBER									
	15	16	17	18	19	20	21	24	25	26
Duration ligation in days	56	29	15	86	168	196	205	256	226	220
Right middle lobe (preoperative control)	60	55	8	77	24	35	12	17	17	13
Right lower lobe (ligated artery)	66	49	11	66	9	33	12	25	29	13
Left lower lobe (postoperative control)	60	30	12	66	11	33	12	20	41	14

TABLE 2. Volume and weight relationships

	DOG NUMBER						
	18	19	20	21	24	25	26
Duration (days)	86	168	196	205	256	226	220
Volume collapsed (cc.)							
Right lower lobe	—	—	—	—	35.5	44.5	45
Left lower lobe	—	—	—	—	35.0	45.0	47
Volume expanded (cc.)							
Right lower lobe	—	—	—	150	175	—	205
Left lower lobe	—	—	—	200	235	—	335
Inflation pressure (cm. H_2O)	—	—	—	15	26	—	26
Weight (grams)							
Right lower lobe	15.3	25	20	27	18.3	35.5	22.0
Left lower lobe	16.4	20	20	28	17.9	35.5	22.3

Dashes indicate data not collected for individual experiment.

Table 2 summarizes the comparative weight, collapsed volume and expanded volume in certain later experiments, where these observations were collected. In the earlier experiments they were not done since interest had not yet centered on this phase of the problem.

In every instance at autopsy, the right lower lobe with ligated pulmonary artery was paler in color, slightly denser in texture and somewhat more resistant to inflation. The relative weights and *collapsed* volumes were, however, within expected limits of normal.

Adhesions between visceral and parietal pleura, and between lobes on the side of operation, were variable in extent, nearly absent in some specimens, and dense

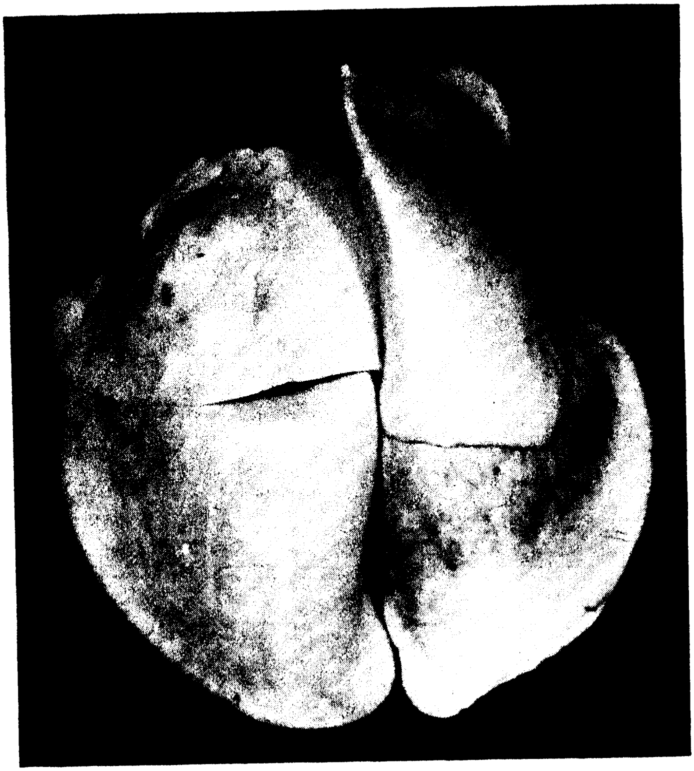

FIG. 1. PHOTOGRAPH OF EXCISED INFLATED LUNGS, dog 21. Note relative decrease in degree of inflation in right lower lobe and subpleural capillary enlargement adjacent to the fissure.

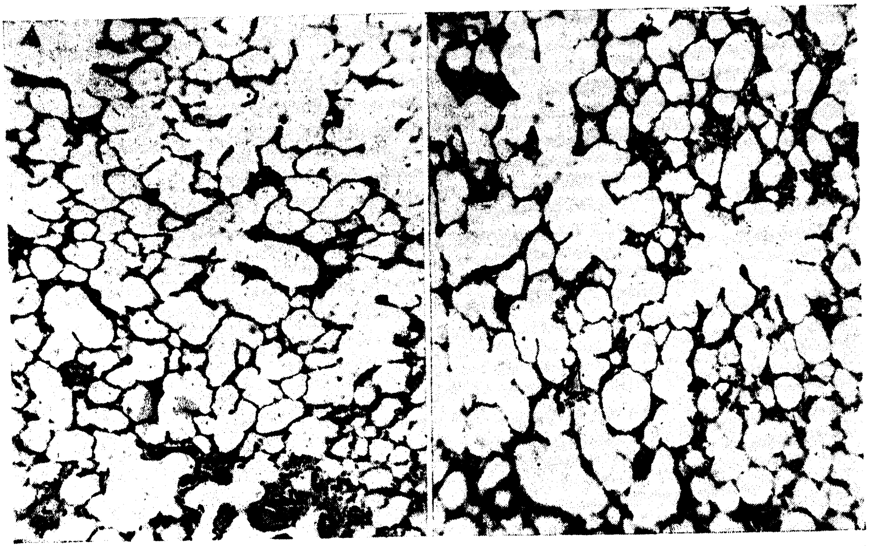

FIG. 2. A. PHOTOMICROGRAPH: right lower lobe section. *H* and *E* stain, 205 days after ligation of pulmonary artery to the lobe. Dog 21.

B. CONTROL SECTION from left lower lobe, same subject.

along the scars of the thoracotomy wound in others. Several specimens showed significant dilatation of the bronchial arterial branches and of the subpleural vessels (fig. 1), suggesting collateral channels.

Histologically, the architecture of the lobe deprived of pulmonary arterial circulation was well preserved (fig. 2a). The capillaries of the alveolar septa were reduced in size, and fewer red cells were in evidence, although some were present. Connective tissue stains showed a slight increase in fibrous tissue in the interlobular septa on the side of ligation.

There was no evidence at autopsy of atelectasis in any lobes, and no instance of gangrene.

DISCUSSION

The failure of pulmonary histamine levels to be significantly altered by long-standing deprivation of pulmonary blood flow renders unlikely the hypothesis that the maintenance of high histamine levels in the lung is dependent on a filtering or clearing function exerted by the lung parenchyma on the venous blood of the pulmonary artery. In other words, the 'storage' theory is given no support by these findings.

The stability of pulmonary histamine levels after pulmonary artery ligation is also in agreement with failure to demonstrate changes in vivo under other circumstances in which acute or chronic injury is done the *living* lung. The present authors have previously demonstrated (3) that experimentally induced lobar pneumonia in dogs does not alter the histamine levels if account is taken of the change in lung weight during consolidation. In some as yet unpublished data on the effect of a war gas (phosgene) a similar conclusion was reached. These observations support the hypothesis that histamine is firmly bound at some site in the lung tissues and is not readily released, short of actual tissue destruction.

The preservation of a nearly normal architecture and histology for many months after interruption of the pulmonary artery supply to a lobe is in agreement with the known function of the bronchial circulation in the nutrition of the pulmonary parenchyma. The persistence of a normal lobar weight and normal collapsed lobar volume demonstrates an absence of significant atrophy or atelectasis, while the reduction in expanded lobar volume after pulmonary artery ligation (as compared with the contralateral normal lobe) points to a loss in elasticity. This results from an increase in collagen in the interlobular tissues, which has been demonstrated by connective tissue stains.

SUMMARY

Operative interruption of the pulmonary arterial flow to the right lower lobes in ten dogs for periods ranging from two weeks to eight months produced no significant change in the levels of extractable pulmonary tissue histamine, as compared with preoperative and postoperative controls from the same animals. These findings suggest that the high concentration of extractable histamine nor-

mally present in the lungs is not the result of a pulmonary filtration, fixation and storage mechanism.

The structural integrity of the lobes deprived of pulmonary arterial blood supply has incidentally been studied. The weight and collapsed volume remain normal, while the expanded volume is diminished.

REFERENCES

1. Dragstedt, C. A. Quart. Bull. Northwestern Univ. Med. School **14:** 283, 1940.
2. Gilman, A. and Lindskog, G. E. Yale J. Biol. Med. **14:** 387, 1942.
3. Simmonds, L. Unpublished Thesis, Yale University School of Medicine, 1941.

BRAIN AND MUSCLE POTASSIUM IN RELATION TO STRESSFUL ACTIVITIES AND ADRENAL CORTEX FUNCTION[1]

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Pincus and Hoagland (1) and Pincus (2) have reported that a wide variety of stresses such as flying airplanes, the taking of examinations, exposures to heat or to cold and factory work result in enhanced output of adrenal cortical hormones. In addition, it appeared that one's fatigability, among other things, is related to the responsiveness of the adrenal cortex to stress.

The administration of the nontoxic, synthetic steroid Δ5-pregnenolone to a group of pilots resulted in a reduced output of urinary 17-ketosteroids per increment of flight stress (3), and since low increments in excretion under stress had been found to correlate with greater resistance to fatigue, we found this of special interest. A number of studies were carried out by Pincus and Hoagland (3) by using subjects operating a pursuit meter when taking placebos and when taking Δ5-pregnenolone, and in addition, investigations were made of the daily piecework production of three groups, comprising a total of 281 factory workers (4, 5). Significant improvement in performance of fatiguing psychomotor tasks was found when the workers were taking 50 to 100 mgm. per day of Δ5-pregnenolone in contrast to placebos. This improvement in fatiguing psychomotor performance only occurred in situations which were really stressful and in which the subjects were well motivated.

The mechanism of the antifatigue action of pregnenolone is of interest. Adrenal steroids with an oxygen in the number 11 position are known to be primarily concerned with gluconeogenesis and in connection with this produce a fall in circulating lymphocytes (6). Steroids of the type of desoxycorticosterone (lacking an oxygen in the number 11 position) are primarily concerned with the regulation of salt and water metabolism (4, 10, 11, 30). Δ-5-Pregnenolone (hereafter referred to as pregnenolone) structurally resembles desoxycorticosterone more than it does other known adrenal steroids, since it also lacks an oxygen at its number 11 carbon atom.[2] We have, moreover, found that its

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[1] This work was performed under Contract N6ori-197 of the Office of Naval Research. It was also aided by a grant from the Schering Corporation.

[2] Selye (see 26 for citations) found pregnenolone to have no depressive action on the pituitary. Pregnenolone differs from desoxycorticosterone in that its number 21 carbon atom is in the form of a methyl group instead of an alcohol group and it contains an hydroxyl group instead of an oxygen atom on the number 3 carbon. It also has a double bond between carbons 5 and 6 instead of between carbons 4 and 5, as in the case of desoxycorticosterone.

injection into rats does not alter the circulating lymphocyte count, nor does its ingestion in doses of 100 mgm. per day by mouth modify sugar tolerance curves in man (unpublished). It thus seemed to us likely that pregnenolone might play a role in regulating sodium and potassium in tissues. Since potassium is especially important in conduction of nerve impulses and in their synaptic transmission, and since steroids of the type of desoxycorticosterone regulate potassium metabolism, it was thought that an investigation of the possible influence of pregnenolone and of desoxycorticosterone on potassium metabolism, especially of brain but also of muscle, would be of interest.

METHODS

Brain and muscle potassium values have been studied under a variety of experimental conditions in 300 male rats weighing between 100 and 140 grams. Groups of matched rats fed on Purina stock diet were compared as follows: normal rats vs. adrenalectomized rats, adrenalectomized rats vs. adrenalectomized rats injectioned with pregnenolone, normal rats vs. those given desoxycorticosterone, normal rats vs. those given pregnenolone, normal rats vs. those stressed by forced swimming for an hour, stressed swimming rats vs. those similarly forced to swim after previous pregnenolone injections. In addition, comparisons were made between brain and muscle potassium in rats stressed by other means.

Most of the analyses were done with a Perkin-Elmer flame photometer (1, 14, 23), although in some of the experiments radioactive potassium, K42, was used as a tracer.

In all but a few indicated experiments in which animals were killed by etherization, the rats were rapidly killed by decapitation; control rats alternating in all cases with experimental animals in the sequence of killing. Tissues, brain and muscle, were removed immediately from *alternating* control and experimental animals. The tissues were placed on filter paper on crucible covers and weighed at once in the wet state. Thus, in a typical experiment involving 20 animals, all were decapitated in a span of three or four minutes and all of the 40 muscle and brain samples were excised and weighed within an hour. The alternation of control and experimental sampling is, of course, important to prevent losses of weight by evaporation or other changes which might modify results in favor of either the control or of the experimental group.

The weighed tissue, whole brain or gastrocnemius muscle, was ashed at 550°C. for one and one half hours in a muffle furnace. In tests with K42 the ashing was done on a crucible cover which was then covered with thin tissue paper and inverted over the mica window of our Geiger-Muller counter.

In experiments with the flame photometer, the ash was taken up in 100 cc. of water, diluted one part to three, and aliquots of this sample were then analyzed for their potassium content and compared to frequently checked standard solutions. In all of the analytical procedures, we alternated control and experimental samples in making our determinations.

In experiments with K42, our results have been expressed as counts per minute per gram of wet tissue and in the flame photometer experiments as mgm. of potassium per gram of wet tissue. In the section on "Discussion" our reasons for using a wet weight basis will be considered. In the final results, we have expressed the percentage change of brain and muscle potassium concentrations produced by the various experimental procedures and calculated the statistical significance of the differences.

RESULTS

Effects of adrenalectomy and of pregnenolone on brain and muscle potassium. Data were obtained on 38 male rats. Thirteen of them were used as normal controls, and nine additional unoperated animals were given 0.5 mgm. per day of pregnenolone acetate in oil subcutaneously for three days prior to sacrifice. Sixteen rats were adrenalectomized four days before sacrifice, and six of these were given daily injections of pregnenolone as in the case of the nine unoperated animals. The adrenalectomized rats were not compensated with NaCl.

All of the 38 rats were injected intraperitoneally with 3 cc. of a solution containing radioactive potassium (K42)[3] 36 hours before sacrifice, a time interval adequate for the tracer to become distributed throughout the tissues according to data of Noonan, Fenn and Haege (22). The K42 solution contained 114 milliequivalents of K per liter as KCl at a pH of 7. The half life of K42 is 12.4 hours and when it was received from the Clinton pile the activity was such that a 3.0 cc. injection gave a count 36 hours later of approximately 6000 counts per minute per gram of wet tissue.

The rats were killed by etherization and the whole brain and one gastrocnemius muscle were removed from each animal. The tissue specimens were ashed on individual crucible lids and the counts were obtained on each of the individual specimens. Table 1 shows the results.

From the data it may be seen that adrenalectomy increases the concentration of muscle potassium by 37.5 per cent over that of normal animals. Harrison and Darrow (15) and Buehl and Turner (2) have also found increased muscle potassium in rats following adrenalectomy. According to Harrison and Darrow, rats, in contrast to dogs, cats and man, show little or no change in intracellular-extracellular water distribution in uncompensated adrenal insufficiency. We know of no work on the potassium content of brain in adrenalectomized animals. Our data show an increase of 24.0 per cent in the concentration of brain potassium four days after uncompensated adrenalectomy in contrast to an increase of 37.5 per cent in that of muscle.

In our experiments, it may be seen from table 1 that pregnenolone does not affect the potassium concentration of brain or muscle in normal rats but does prevent the marked rise in brain and muscle potassium concentration in adrenalectomized rats. Thus brain potassium concentration increases only 14.0 per cent (instead of 24.0 per cent) and muscle potassium 25.5 per cent (instead of 37.5 per cent) in adrenalectomized rats protected by pregnenolone. All of these differences are statistically significant.

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[3] The K42 was obtained from the Clinton pile through the Atomic Energy Commission.

Effects of desoxycorticosterone acetate (DCA) (Schering) on brain and muscle potassium and sodium. While pregnenolone does not affect the potassium concentration of brain and muscle of normal unstressed rats, desoxycorticosterone acetate (DCA) injected in the same amount and under the same conditions results in a small but significant lowering of muscle potassium concentration, although it has no significant effect on brain potassium. DCA is the adrenal steroid primarily effective in the control of sodium and potassium metabolism (4, 10; see Goodman and Gilman (11), Thayer (30) for reviews).

TABLE 1. COUNTS PER MINUTE PER GRAM WET WEIGHT TISSUE

	1	2	3	4	Comparing columns 2 to 1	Comparing columns 4 to 3	Comparing columns 3 to 1
	Normal control	Normal control pregnenolone injected	Adrenalectomized rats	Adrenalectomized pregnenolone injected			
Brain	4897 ±124	4802 ±200	6075 ±308	5460 ±360	−2% P not signif.	−10% P<0.01	+24% P<0.01
Muscle	5943 ±143	6123 ±108	8172 ±244	7198 ±381	+3% P not signif.	−12% P<0.01	+37.5% P<0.01

TABLE 2. MGM. POTASSIUM AND SODIUM PER GRAM WET WEIGHT TISSUE

	9 NORMAL RATS		9 DCA-INJECTED RATS		% CHANGE INJECTED VS. CONTROLS	% CHANGE INJECTED VS. CONTROLS
	K	Na	K	Na	K	Na
Brain	3.138	1.156	3.108	1.138	1.0% loss P not signif.	1.5% loss P not signif.
Muscle	3.164 ±0.06	1.002 ±0.01	2.967 +0.05	1.045 ±0.025	6.2% loss P = 0.02	4.0% gain P not signif.

Nine rats were injected with DCA in oil (0.5 mgm. per day for five days) prior to sacrifice, and nine paired rats were kept as controls. The animals were killed by decapitation and the brain and one gastrocnemius muscle from each rat were analyzed for potassium and sodium with the flame photometer. Table 2 shows the results.

The only significant effect is a decline in potassium concentration in muscle of 6.2 per cent. The 4.0 per cent gain in muscle sodium is not statistically significant. Miller and Darrow (4, 21) have also reported a loss of potassium from muscles of normal rats given DCA.

We have not examined the effect of DCA on brain and muscle potassium of adrenalectomized rats. Harrison and Darrow (15) have shown that adrenal cortical extract reduces the high muscle potassium of adrenalectomized rats to normal, presumably via its DCA-like components.

Stress of prolonged swimming in relation to brain and muscle potassium and the effect of pregnenolone on tissue potassium. From the data of table 1, it is clear that pregnenolone in the small amounts we used lessens the excessive accumulation of potassium in both brain and muscle resulting from adrenalectomy. In this way it acts like adrenal cortical extract which regulates electrolyte balance by way of its desoxycorticosterone-like content.

Because of our studies of psychomotor fatigue in man in relation to pregnenolone, we were particularly interested in effects of fatiguing stresses on brain and muscle potassium and possible influences of pregnenolone on tissue potassium in relation to stress. Was it possible that fatiguing stresses might modify tissue potassium and that pregnenolone would prevent such changes?

Miller and Darrow (21) stressed rats by forced swimming for periods ranging up to an hour. They found no loss of potassium from muscle as a result of the exercise, although it has been well established that isolated muscle stimulated to contract through its nerve loses potassium. (See Fenn (9) and Darrow (5) for reviews.)

We have stressed rats by forced swimming at 28°C. for an hour. In each experiment all rats, both of the experimental and control groups, were killed immediately after the swim by decapitation, and brain and muscle potassium were determined by the flame photometer. The variability of tissue potassium was found to be so great that it was necessary to use considerably larger numbers of animals than had been used by Miller and Darrow before statistically significant differences were obtained. As may be seen from table 3, there is a small but significant decrease in the concentration of potassium in both brain and muscle of the swim-stressed rats compared to the controls, i.e., 4.4 per cent decrease in brain and 6.1 per cent decrease in muscle.

A comparison of data from tables 2 and 3 show different values of potassium in the two groups of normal control rats. In each experiment we have used control rats to match against our experimental groups, since over the months occupied by the work we have found from time to time occasional differences in mean potassium analyses of tissues in different groups of rats. When the data of table 2 were obtained, our rat colony was suffering from a mild form of infectious diarrhea with some loss of appetite, and we think it likely that potassium loss in the stool may account for the low values of the experimental and control groups of table 2, in contrast to the higher values later obtained as shown in tables 3 and 4. Our interest throughout has been in the comparative values of potassium in relation to matched groups of rats and we have not attempted to do potassium balances. The data of tables 3 and 4 show better representative values of typical potassium levels than do those of table 2, but the comparison of relative potassium values of control and experimental animals we believe to be equally valid for the data of each table.

The effects of pregnenolone on potassium changes resulting from the stress of swimming is also shown in table 3. In these experiments a group of rats were injected with 0.5 mgm. of pregnenolone per day for four days prior to the swim. These rats, together with an uninjected matched control group, were all rapidly

TABLE 3. Mgms. potassium/gram wet weight tissue

	1	2	3			
	Normal control	Swim stress	Swin stress pregnenolone-injected	Comparing column 2 to 1	Comparing column 3 to 2	
Brain	3.530 ±.026	3.376 ±.036		−4.36% P < 0.01		54 swim stress vs. 65 normals. Combination of 6 experiments.
		3.236 ±0.05	3.336 ±0.05		+3% P = 0.1–0.2	36 swim stress vs. 23 swim pregnenolone-injected. Combination of 2 experiments.
Muscle	3.356 ±.047	3.15 ±.05		−6.14% P < 0.01		32 normals vs. 29 swim stress. Combination of 5 experiments.
		3.198 ±0.067	3.349 ±0.072		4.7% P = 0.1–0.2	26 swim stress vs. 21 swim stress pregnenolone-injected. Combination of two experiments.

TABLE 4. Mgms. potassium/gram wet weight tissue

	1	2		
	DCA-injected	DCA-injected swim stress	Comparing columns 2 to 1	
Brain	3.471 ±0.04	3.579 ±0.03	+3.1% P = 0.1–0.05	1 experiment. 10 DCA-injected animals (0.5 mgm/day for 5 days) vs.
Muscle	3.672 ±0.04	3.396 ±0.06	−7.5% P < 0.01	10 DCA-injected animals (0.5 mgm/day for 5 days) swim stressed.
Mgms. sodium/gram wet weight tissue				
Brain	1.176	1.182	+0.5% P not signif.	
Muscle	0.776 ±0.016	0.845 ±0.02	+9% P = 0.02–0.05	

decapitated at the end of the swim of the experimental group, and the brain and muscle potassium values were individually determined with the flame photometer.

Pregnenolone-injected rats that were forced to swim have a 3.0 per cent higher potassium concentration in their brains and 4.7% higher potassium concentration in their muscles than have rats forced to swim but not previously injected with pregnenolone. These differences are in the right direction if pregnenolone prevents loss of potassium due to the stress. However, the results are not statistically significant. The P value of 0.1 to 0.2 indicates that there is a ten to 20 per cent likelihood that the differences are due to chance.

Sodium values were also obtained in the experiments involving swimming, but they were highly variable. No significant differences were observed in the sodium content of the brains of rats forced to swim as compared to unstressed controls, but a significant 23 per cent increase in the concentrations of muscle sodium (P ± 0.01) was found in rats forced to swim corresponding to a decline of 6.1% of potassium concentration in this group.

If the decline in concentration of potassium in brain and muscle is important in the development of fatigue, and if pregnenolone tends to prevent this decline, it should follow that pregnenolone-injected rats would be able to swim longer than controls.

The time that a rat can swim before exhaustion is dependent upon the temperature of the water. While most rats are not exhausted after an hour's swim at 35°C., few rats fail to drown in half an hour in water at 15°C.

Eighteen rats were forced to swim in a bath at 22°C. Nine of them had previously been injected for five days with 0.5 mgm. per day of pregnenolone in oil. The nine control rats were given placebo injections of oil primarily so as to be comparable in buoyancy to the pregnenolone group. The period from the start of the swim to drowning was measured. The average length of swim of the pregnenolone group was 56 minutes, and that of the control group 41 minutes, but the variability was so great (from 14 minutes to 180 minutes) that this result is not statistically significant. We estimated that at least several hundred rats would require drowning adequately to answer this question and our colony could not at the time afford this attrition rate.

Effect of DCA on potassium metabolism under conditions of swim-stress. From the data of table 2, it may be seen that normal unstressed rats injected with DCA show a reduction of 6.2 per cent in the concentration of muscle potassium.

Table 4 indicates that DCA injected into rats at a rate of 0.5 mgm. per day for four days prior to forced swimming produces a drop in the concentration of muscle potassium following the swim below the levels found in uninjected swim-stressed rats. There appears to be no compensatory effect of the hormone in overcoming the decline in potassium concentration from muscle due to stress as is suggested from the results with pregnenolone. Accompanying the decline in potassium concentration is a significant (9 per cent) rise in the concentration of muscle sodium.

Animals previously injected with DCA and forced to swim, however, appear to show less of a decline of brain potassium concentration (by 3.1 per cent) than uninjected swim-stressed rats, and this difference is just barely significant. No difference in brain sodium is seen in the two groups. Pregnenolone and DCA thus both appear to act to prevent the lowering of potassium concentration in brains of stressed animals. Pregnenolone also prevents decrease in concentration in potassium from muscle, while DCA accentuates the decrease. But these effects are small and some of them may be due to chance. Figure 1 summarizes the data of the four tables.

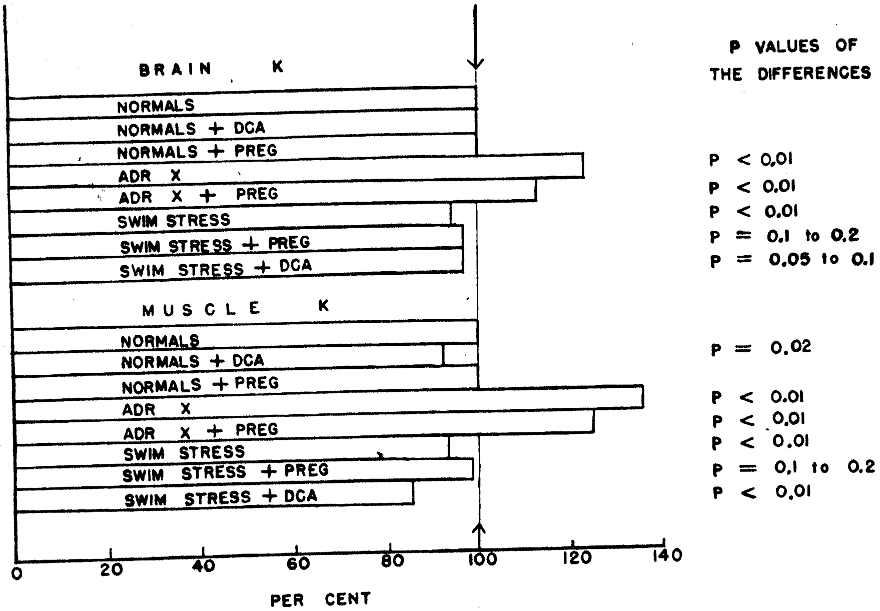

Fig. 1. Summary of the data of the four tables. Per cent change (wet weight basis) of potassium concentration in brain and muscle under various experimental conditions.

Other stresses. Work from our laboratory (7) has demonstrated that exposures of rats to cold and also to the stress of tying them to a grid markedly excites the adrenal cortex as indicated by pronounced lymphocytopenia. This lymphocytopenia is absent in comparably stressed adrenalectomized rats. Dougherty and White (6) have demonstrated that lymphocytopenia occurs when adrenal cortical extract or adrenocorticotrophin is injected into normal rats, and that adrenocorticotrophin fails to produce lymphocytopenia in adrenalectomized rats. More specifically, they have shown that the drop in lymphocytes results from the action on lymphoid tissue of 11-oxygenated, 'sugar' steroid hormones from the adrenal cortex.

In view of the excitation of the adrenal cortex by cold stress and grid stress, we felt it would be interesting to examine brain and muscle potassium and sodium in relation to these stresses. Twenty rats were exposed in a cold room to a temperature of 2°C. for four hours, immediately decapitated and their brains and muscles analyzed for potassium and sodium with the flame photometer. Twenty paired rats, kept at room temperature, were used as controls. No significant differences were found in brain or muscle potassium and sodium between the experimental and control groups.

Ten rats were tied to a wire grid for two hours at room temperature and their brain and muscle sodium and potassium values were compared to those of the unstressed control rats. We found no differences in brain or muscle sodium or potassium between the two groups.

Our failure to find changes in the concentration of tissue potassium with cold and grid stresses in contrast to our findings with the stress of swimming suggests that a possibly different type of excretion of adrenal cortical hormones may be involved in the two cases. This is borne out by our observations (to be published elsewhere) that the stress of swimming produces marked lymphocytosis in contrast to the equally marked lymphocytopenia resulting from cold or tying to a grid. It is possible that different pituitary corticotropins may be involved in the release of different steroid hormones from the adrenal cortex in the case of the different stresses.

DISCUSSION

Zwemer and Truszkowski (31) have emphasized the similarity of symptoms, including fatigue symptoms, between adrenal insufficiency resulting in potassium retention and the symptoms of potassium poisoning. From our experiments there is a reduction of brain and muscle potassium concentration with prolonged activity. Pregnenolone in small doses appears to have a compensatory action on both increased potassium concentration in adrenal insufficiency and decreased potassium concentration due to stress.

This homeostatic action of pregnenolone on potassium, particularly in relation to the brain, is suggestive in connection with the action of pregnenolone in enhancing the efficiency of prolonged psychomotor behavior in man.

We have expressed our potassium analyses on a wet weight basis, despite well established demonstrations by others (5) of changes in water content of muscle with activity. The analysis of some 600 tissue samples has been facilitated by elimination of the operation of drying the tissue, but in addition we have felt that the *concentration* of potassium per gram of tissue, despite its water content, is of as much, if not more, physiological interest than is the amount of potassium per unit weight of solids. Potassium greatly modifies excitability of tissues including nerve, and depending upon concentration gradients, it can either enhance or inhibit excitability (see Fenn (9) for review). Entrance or loss of water modifies potassium concentration as does the loss or entrance of potassium itself.

Fenn (9) has reviewed evidence indicating that nerve together with many other excitable tissues loses potassium during activity, and he has reviewed the demonstrated relationship between the metabolism of potassium and that of acetylcholine in nerve conduction and synaptic transmission. Potassium is highly mobile in protoplasm and nerve cells, like most other cells, contain a high inside concentration of potassium amounting to 10 or 40 times that in the surrounding fluids. Hoagland (17) has demonstrated that the excitability to mechanical stimuli of single nerve endings in the frog skin is determined by the ratio of concentration of potassium inside the fiber to that outside. This ratio of potassium inside to potassium outside (K_i/K_o) appears to result in major part from the intracellular formation of H^+ produced by the metabolic formation of acid substances, especially CO_2 and the exchanges of H^+ for K^+ (See Grundfest (13) for review).

Curtis and Cole (3), following leads from Osterhout's (24) work with 'impaled' *Valonia* plant cells, succeeded in pushing a microelectrode into the axoplasm along the axis of a giant nerve fiber of the squid, so that the tip was just opposite the outside electrode. The membrane potential then measured was 50 mV. Upon raising the outside concentration 18-fold (making K_i/K_o zero), the potential was reduced to zero, and upon raising it 40-fold, the potential was reversed 15 mV. Similar results have been obtained by Graham, Carlson and Gerard (12) for single frog muscle fibers.

Since the electrical properties of the cell depend upon this ratio rather than upon the absolute potassium content, and since K_i/K_o can be modified by movement of either water or potassium, we have expressed our results on a wet weight percentage basis rather than on a dry weight basis. There seems to us to be little choice between expressing potassium values on a fat-free dry weight basis or on a wet weight basis. K_i/K_o is lowered if potassium is lost from the cell. It is also lowered if water enters and lessens the internal concentration. Since little is known about the distribution of intracellular, extracellular and bound water in muscle under different experimental conditions, and since nothing is known about this distribution in brain, we believe that the wet weight concentrations of potassium are as meaningful as a first approximation as are concentrations expressed on a dry weight basis. It follows, of course, that neither is necessarily meaningful.

Recently Hodgkin (18) found that small changes in external potassium concentration caused large and rapidly reversible changes in the conductance of crab nerve membranes. Hodgkin and Huxley (19) have calculated that 1.7×10^{-12} moles of potassium leak through one square cm. of crab nerve membrane per impulse and that 3×10^{-10} mol. cm.$^{-2}$ sec.$^{-1}$ are reabsorbed when the external concentration of potassium is three times its normal value.

Potassium in nerve, because of its high mobility and consequent internal accumulation, is important in determining nerve excitability and in the conduction and transmission of nerve impulses. The fact that other cations, notably rubidum, not present in body fluids in appreciable amounts, affect the electrical behavior of nerve in a way similar to that of potassium (20) does not militate, in our opinion, against this view. Homeostasis in the concentration of brain potassium would thus be expected to be associated with optimal brain functioning, and it is not unreasonable to expect modification of the conduction of impulses in the central nervous system if brain potassium concentration is either elevated or depressed.

Heppel (16) studied changes in water, chloride and potassium content of rat gastrocnemius muscles when made to contract by electrical stimulation of the sciatic nerve. He confirmed previous work of Fenn and co-workers (9) on muscles of rats and other animals, indicating that stimulation of muscles results in a loss of potassium in exchange for sodium and a gain in NaCl and water. Heppel found that the loss of potassium on stimulation is greater the greater the duration of contraction up to 30 minutes, with a decline in rate of loss after this time. The changes in water and chloride were maximal after five minutes of stimulation and much reduced at the end of an hour. While the water variations parallel those of chloride, potassium loss was not correlated with the water changes. The feeding of diets low in potassium had little or no effect on the potassium changes during stimulation.

Fenn (8) earlier had studied the effect of voluntary contraction of muscles in nine rats forced to swim to exhaustion. He cut one sciatic nerve in each animal and compared the potassium and water content of the active and inactive leg muscles. He summarized his results as follows:

> "Rats with one sciatic nerve cut were exercised by swimming. The muscles sampled immediately afterwards and analyzed showed that voluntary contractions produced an increase of water content and a decrease of potassium (on a dry weight basis). In general the muscles of rats which swam the longest and were least quickly fatigued lost the most potassium but gained the least water. Muscles sampled a few days after denervation showed a slight gain in potassium, presumably because of the lack of activity."

Miller and Darrow (21) were unable to find any loss of potassium from rat muscles (dry weight basis) after the voluntary exercise of forced swimming, although an elevated serum potassium was found after the exercise. In one group of experiments they lowered the potassium content of muscle potassium in rats by feeding them on a potassium-free diet, and in another group they raised the potassium content by injecting the animals with potassium chloride. Within wide limits they found that potassium in muscle cells does not limit the capacity of rats to swim continuously for 60 minutes. They criticized Fenn's findings on the grounds that his reported post-swim loss of potassium from active muscles was not statistically significant.

Miller and Darrow (21) found that the injection of two to 4 mgm. per day of DCA for nine to ten days in eight rats gave an average muscle potassium content of 40.5 mM potassium per 100 gram fat-free solid, which contrasted with a mean value of 48.8 in a group of 13 uninjected rats. The difference of 8.3 mM of potassium corresponds to a loss of 17.0 per cent in muscle potassium in the DCA-injected group, and this may be compared to the 6.2 per cent loss of potassium we found on a wet weight basis after injecting 0.5 mgm. per day of DCA for five days (table 2).

Miller and Darrow (21), however, report that the stress of swimming raises the level of potassium in muscles of DCA-injected rats, and this appears to be contrary to our results since we found an apparent loss of muscle potassium in DCA-injected rats that were forced to swim. In the case of brain, however, DCA tends to restore to a normal level the decrease in concentration of brain potassium resulting from forced swimming (table 4), and pregnenolone appears to do this also for muscle as well as for brain (table 3).

Our results show that both brain and muscle display a significantly decreased concentration of potassium after forced swimming, and thus we are in agreement with Fenn's finding regarding changes in muscle potassium with voluntary exercise. Our findings appear to be in disagreement with the results of Miller and Darrow in this regard, although it was necessary to use many more animals than other investigators had used to demonstrate the change in potassium concentration with statistical validity. Since we have expressed results on a wet weight basis, it is possible the apparent potassium loss we note with exercise in muscle is due to a gain in water. For reasons discussed above we believe that potassium concentration changes, whether they be due to gain or loss of either water or potassium, is of primary physiological interest.

SUMMARY

1. Studies of the concentration of brain and muscle potassium of 300 rats were made under a variety of experimental conditions.

2. Small (0.5 mgm. per day) doses of desoxycorticosterone or of Δ5-pregnenolone did not affect brain potassium in normal rats; desoxycorticosterone, but not Δ5-pregnenolone, reduced the concentration of muscle potassium by 6.2 per cent.

3. Uncompensated adrenalectomy increased the concentration of brain potassium by 24.0 per cent and of muscle potassium by 37.5 per cent.

4. Δ5-Pregnenolone (0.5 mgm. per day) lessened the increase in concentration of brain potassium in adrenalectomized rats by 10.0 per cent and of muscle potassium concentration by 12.0 per cent.

5. The stress of prolonged swimming reduced the concentration of brain potassium by 4.4 per cent and that of muscle by 6.1 per cent. These, together with the foregoing results, were all statistically significant ($P < 0.01$).

6. There was a suggestion that doses of 0.5 mgm. per day of both Δ5-pregnenolone and desoxycorticosterone tended to prevent the loss of brain potassium due to prolonged swimming, although the results were not statistically significant. (P approximately 0.1.)

7. Δ5-pregnenolone also tended to prevent the decrease of potassium concentration in muscles of animals forced to swim, but the P value was not significant (0.1 to 0.2).

8. Desoxycorticosterone significantly augmented the decrease of concentration of muscle potassium in swim-stressed rats..

9. The results are discussed in relation to effects of potassium on tissue excitability and to the antifatigue action of pregnenolone.

REFERENCES

1. Barnes, R. B., D. Richardson, J. W. Berry and R. L. Hood. Ind. & Eng. Chem. Anal. Ed. **17**: 605, 1945.
2. Buell, M. V. and E. Turner. This Journal **134**: 225, 1941.
3. Curtis, H. J. and K. S. Cole. J. Cell. & Comp. Physiol. **19**: 135, 1942.

4. Darrow, D. C. and H. C. Miller. J. Clin. Invest. **21**: 601, 1942.
5. Darrow, D. C. Ann. Rev. Physiol. **6**: 95, 1944.
6. Dougherty, T. F. and A. White. Endocrinology **35**: 1, 1944.
7. Elmadjian, F. and G. Pincus. Endocrinology **37**: 47, 1945.
8. Fenn, W. O. This Journal **120**: 675, 1937.
9. Fenn, W. O. Physiol. Revs. **20**: 377, 1940.
10. Ferrebee, J. W., Parker, D., Carnes, W. H. Gerrity, M. K., Atchley, D W. and R. F. Loeb. This Journal **135**: 230, 1941.
11. Goodman, L. and A. Gilman. Macmillan: The Pharmacological Basis of Therapeutics. New York, 1941.
12. Graham, J., G. R. Carlson and R. W. Gerard. Federation Proc. **1**: 31, 1942.
13. Grundfest, H. Ann. Rev. Physiol. **9**: 477, 1947.
14. Hald, P. M. J. Biol. Chem. **167**: 499, 1947.
15. Harrison, H. E. and D. C. Darrow. J. Clin. Invest. **17**: 77, 1938.
16. Heppel, L. A. This Journal **128**: 440, 1940.
17. Hoagland, H. Cold Spring Harbor Symp. Quant. Biol. **4**: 347, 1936.
18. Hodgkin, A. L. J. Physiol. **106**: 319, 1947.
19. Hodgkin, A. L. and A. F. Huxley. J. Physiol. **106**: 341, 1947.
20. Lorente de Nó, R. J. Cell. & Comp. Physiol. **29**: 207, 1942.
21. Miller, H. C. and D. C. Darrow. This Journal **132**: 801, 1941.
22. Noonan, T. R., W. O. Fenn and L. Haege. This Journal **132**: 474, 1941.
23. Overman, R. R. and A. K. Davis. J. Biol. Chem. **168**: 641, 1947.
24. Osterhout *et al*. J. Gen. Physiol. **11**: 103, 1927.
25. Pincus, G. and H. Hoagland. J. Aviation Med. **14**: 173, 1943.
26. Pincus, G. and H. Hoagland. J. Aviation Med. **15**: 98, 1944.
27. Pincus, G. and H. Hoagland. Psychosomatic Med. **7**: 342, 1945.
28. Pincus, G., H. Hoagland, C. H. Wilson and N. J. Fay. Psychosomatic Med. **7**: 347, 1945.
29. Pincus, G. Academic Press: Recent Progress in Hormone Research. New York, **1**: 123, 1947.
30. Thayer, S. A. Academic Press: Vitamins and Hormones. New York, **4**: 311, 1946.
31. Zwemer, R. L. and R. Truszkowski. Endocrinology **1**: 40, 1937.

CONDUCTION RATES AND DORSAL ROOT INFLOW OF SENSORY FIBERS FROM THE KNEE JOINT OF THE CAT[1, 2]

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The analysis of the neural control of skeletal muscle as it acts on diarthrodial joints has been carried to a very high degree by Sherrington and many subsequent workers, but surprisingly little attention has been paid to the innervation of joints themselves, either from an anatomical or physiological standpoint. This is particularly astonishing in view of the incidence of crippling joint disorders in which nerves must be implicated since severe pain is a common symptom. Yet it is not known just where such pain arises. Raszeja and Billewicz-Stankiewicz (10) described areas on the medial and lateral sides of the rabbit knee joint, the stimulation of which, with a faradic current of two seconds' duration, was followed by responses which indicated that the stimulus was painful. Leriche (9) on the other hand stated that an anesthetic injected into the cavity of a painful joint had no effect on the pain, but when it was injected into the external part of the capsule and around the joint, the pain was relieved. Our knowledge of the transmission of pain from joints is no more precise than indicated in these two reports.

Position sense is a useful test in neurological examinations and undoubtedly an important quality in postural and locomotor mechanisms. It seems highly probable that stimulation of joint receptors is one of the necessary components. This was borne out by Stopford (12) in his studies of peripheral nerve lesions. He pointed out that the so-called cutaneous nerves of the fingers give branches to the interphalangeal joints. When these nerves are destroyed the patient can still detect joint movements passively induced by the examiner, but his localization of direction, degree and site of movement is greatly impaired. Stopford felt that recognition of movement as such could result from changes in tension of tendons, the nerves to which were still intact. Presumably, then, joint endings are stimulated during movement. Barnes (2) recorded potentials from sensory nerves of crustacean limbs while the joints of such limbs were being bent. In a search of the literature, however, no reports of similar studies in vertebrates were found.

There is considerable clinical evidence that the central pathway for position sense is by way of the dorsal funiculus, medial lemniscus and internal capsule, but there have been no experimental studies of any of the possible central connections of these receptors, aside from some studies by Adrian on cerebellar action potentials (1). He recorded potentials during various types of peripheral stimuli, among which were joint movements.

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[1] The initial phases of this work were presented at the Annual Meeting of the American Association of Anatomists at Montreal, April 1947.

[2] Aided by a grant from the Division of Research Grants and Fellowships, National Institute of Health, U. S. Public Health Service.

There are only scattered reports relative to reflexes in which these nerves and their terminals may partake. Harrison *et al.* (7) and Comroe and Schmidt (4) in their studies of respiratory mechanisms concluded that impulses from joint receptors initiated reflex increases in respiration. There have been no studies of possible reflex effects on skeletal muscle.

Our lack of knowledge is further emphasized by the fact that, so far as human joints are concerned, no one has determined exactly what kinds of nerve endings are present. The evidence that Pacinian corpuscles are found in joint capsules is quite unsatisfactory (5).

There are other points which could be made but these are enough to indicate how little attention has been paid to the functions of normal joints.

Distribution of nerves to the knee joint. An experimental approach to these problems depends upon a rather exact knowledge of the anatomy of joint nerves. Because of this it was felt that studies of the cat would be most satisfactory since it has been shown that the nerves to the knee joint of this animal are distributed in a definite pattern and terminate in various types of receptors in the joint capsule (5). The joint is supplied mainly by the femoral and tibial nerves, and to a lesser extent by the obturator. Two branches to the joint are larger and more constant than any others. One arises from the tibial nerve and, because of its position relative to the knee joint, is designated the posterior nerve. The average nerve contains approximately 170 myelinated fibers which range from two to 16 microns in diameter. The majority are either in a two to five or seven to 10 micron group, the latter containing the greater number of fibers. In addition, there are about 115 nonmyelinated fibers.

The other major branch to the joint is designated the medial nerve. It usually arises from the saphenous branch of the femoral nerve, but occasionally from the obturator, or from both. The average nerve contains approximately 145 myelinated fibers which range from two to 16 microns in diameter. Like the posterior nerve, most of the fibers are either in a two to five or seven to 10 micron group, but in this nerve the two to five micron group contains the greater number of fibers. In addition, there are about 120 nonmyelinated fibers.

METHODS

There were several questions, the answering of which constituted the initial studies of these nerves and their endings. What are the conduction rates of these articular fibers and do they correspond with measured diameters? Are the fibers efferent or afferent, and if the latter, in which dorsal roots do they enter the spinal cord? What are the effective stimuli for the receptors which many of these fibers form?

Adult cats were anesthetized with sodium pentobarbital given intraperitoneally. In approaching the posterior nerve the gastrocnemius muscle was split and the tibial nerve exposed. This was traced towards the popliteal fossa, at the lower part of which there are several branches distributed mainly to the neighboring muscles. The first of these branches is usually the posterior nerve. It descends in the sheath of the tibial nerve for a short distance and then leaves it, running a recurrent course towards the back of the joint capsule, crossing the popliteal vessels as it does so. Its subsequent distribution has already been described in detail (5). The nerve was carefully cleaned of surrounding connective tissue, using glass hooks and probes for most of the dissection. In all cases the tibial nerve was sectioned distal to the origin of this branch so as to facilitate manipulation.

The medial nerve was approached by making an incision in the sartorius muscle in a line parallel to the more deeply placed vastus medialis, along which the nerve descends. The dissection was more difficult since the nerve is smaller than the posterior nerve, often courses as two fascicles and is always closely associated with or actually in the adventitia of the accompanying vessels. Because of these factors, in several dissections the nerves did not survive.

Types of experiments. The following experiments were carried out.

1. Dorsal roots were cut at their entrance into the spinal cord. Potentials were recorded from articular nerves as various roots were stimulated (fig. 1A). At first, several attempts were made to record from dorsal roots as articular nerves were stimulated, but it was found that the large number of fibers derived from areas other than the knee joint shunted out the desired potentials to a large extent. The antidromic method proved much more satisfactory on this account. Furthermore, the absence of a marked connective tissue sheath helped reduce shock artifact.

2. The sciatic and femoral nerves were ligated, cut centrally and all branches severed except the articular nerves. They were then stimulated and muscle contractions in the neighborhood of the joint closely watched for. Next the articular nerves were severed from the capsule, placed on electrodes and potentials recorded during stimulation of the parent trunks (fig. 1B). In this way articular fibers were activated and the recorded potentials could be compared with those obtained by stimulation of some of the same fibers in a particular dorsal root.

3. The articular nerves were severed from the parent trunks and placed on electrodes. Since they were still attached to the joint capsule, the method allowed the recording of any potentials originating from the stimulation of joint receptors.

The animals were kept warm but no special precautions were taken in regard to temperature control. The conduction distances were long and most of either the sciatic or femoral nerves were untouched and therefore at body temperature in those experiments in which conduction rates were measured. Exposed nerves were constantly kept moist with warm Ringer's solution. Under such conditions recordings were carried out over many hours without appreciable changes being observed.

The stimulating electrodes were silver wires, and a silver wire was also used to ground the nerves between stimulating and recording electrodes. The latter were glass tubes filled with Ringer's solution from which woolen wicks protruded and into which dipped chlorided silver wires. The nerves were slung on glass hooks and contact made with the moist wicks of the electrodes. In the earlier experiments, the stimulus was a condenser discharge, the duration of which was approximately one tenth of a millisecond. Later a thyratron control was substituted for the mechanical switching and this allowed considerable variation in both frequency and duration of stimulus. In both cases the stimulus was applied through a shielded transformer which helped reduce shock artifact. The artifact was very marked in several cases, the cause being either a poor contact at the ground electrode or an accidental grounding of some other part of the animal, thus giving a double ground. Sixty-cycle interference often appeared in the latter situation. Both pickup electrodes were above ground, being connected to the input grids of a push-pull amplifier. The input was differentially arranged, with a high resistance common to both cathodes. The amplifier was resistance-capacity coupled with a time constant of about one and one-half seconds. Its high frequency response fell off sharply above 1000 cycles. The output was fed into a cathode ray oscillograph, the sweep of which was tripped by the stimulus.

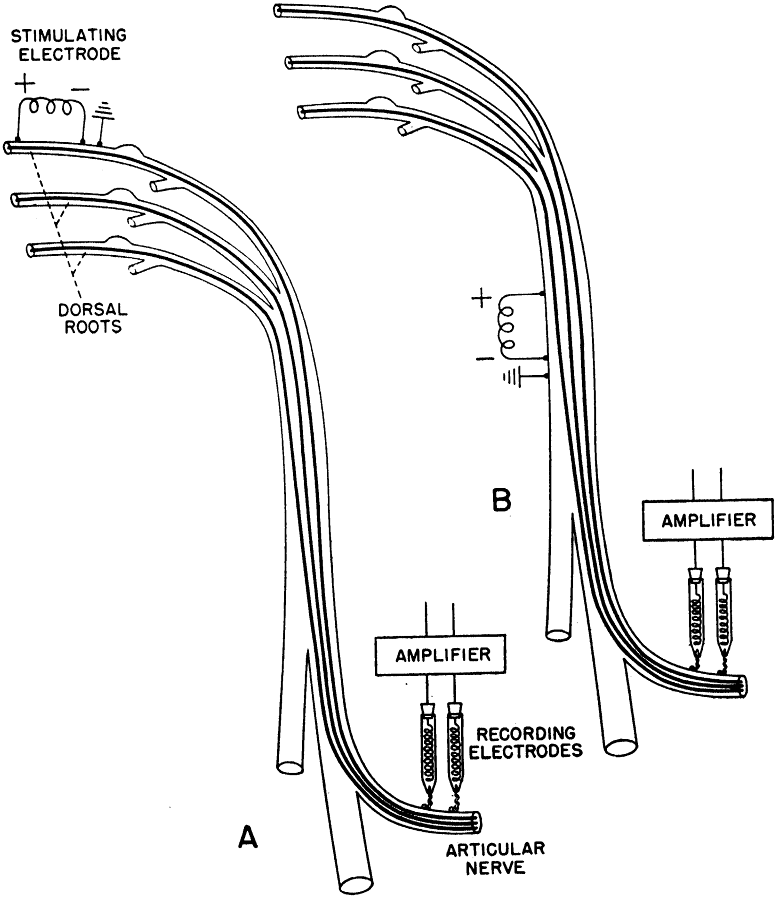

FIG. 1. *A*, DIAGRAM OF THE METHOD USED IN DETERMINING CONDUCTION RATES and dorsal root inflow. The main trunk represented is the sciatic, but the method was the same for the femoral nerve. *B*, Diagram showing how stimulation of the main trunk activates all the myelinated fibers from the articular nerve.

RESULTS

Action potentials were successfully recorded in 19 of 24 cats. In 12 of these 19 the posterior nerve was studied and, in seven of these, photographic records were made. The medial nerve was studied in the remaining seven of the 19 cats and, in five of these, photographic records were made.

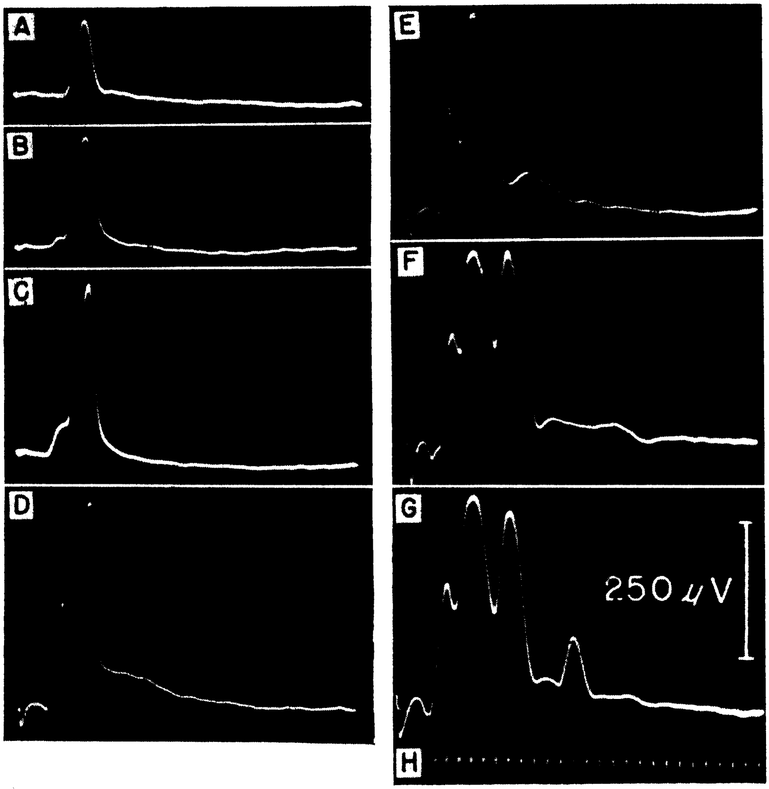

FIG. 2. POTENTIALS RECORDED FROM THE POSTERIOR NERVE DURING STIMULATION of the 7th lumbar dorsal root by the method of figure 1A. *A–G*. Responses at increasing strengths of stimulation, with development of characteristic major deflections. Note that the fast initial component does not have the lowest threshold. It is first seen in *B* at stimulus strength higher than that which elicited the response in *A*. *H*. Time in milliseconds.

Posterior nerve. Potentials were consistently recorded from the posterior nerve on stimulation of the 6th and 7th lumbar dorsal roots, and, in two cases, of the 1st sacral dorsal root as well. The conduction distances ranged from 16.5 to 18 5 centimeters. Consequently, the temporal dispersion was so great that the form of the potentials was often complex. At least two major deflections were usually recorded (fig. 2). In the first there was frequently a small initial component conducting at about 70 to 90 meters a second, but most of the elevation represented fibers conducting at approximately 40 to 60 meters a second. The second major deflection represented fibers conducting at rates of approximately 20 to 40 meters a second. Following this were minor deflections of variable form and amplitude (figs. 2G and 3A), the slowest of which conducted at about ten meters a second.

The recorded potential was much larger when the sciatic was stimulated, since many more articular fibers were activated (fig. 1B). The conduction distance was shorter, so that the major deflections were combined into one, and the more slowly conducting fibers were represented by a somewhat complex and smaller terminal deflection (fig. 3B). When the articular nerve was left attached to the joint capsule during stimulation of the sciatic, muscular contractions were never seen, providing all other branches of the sciatic had been cut.

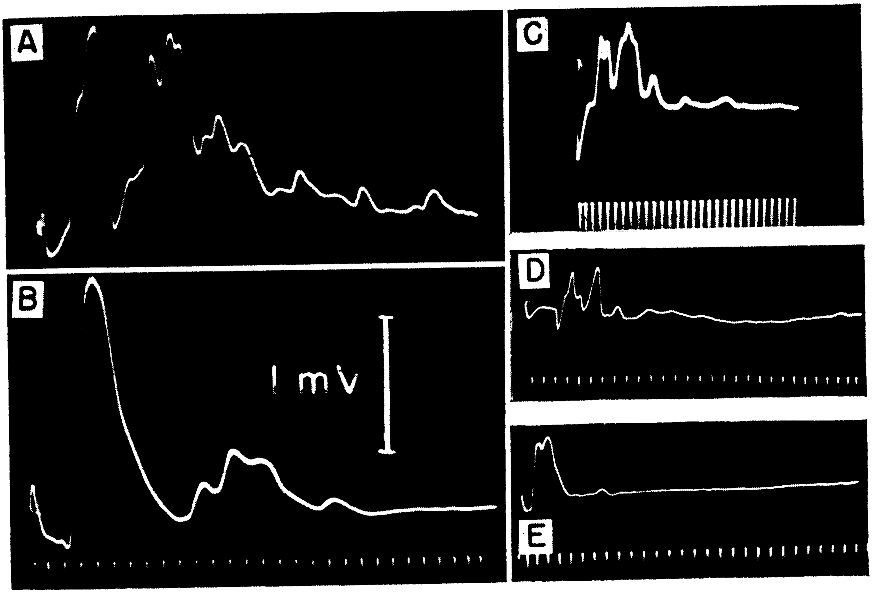

FIG. 3. *A*. POTENTIALS RECORDED FROM THE POSTERIOR NERVE DURING STIMULATION of 6th lumbar dorsal root. Contrast the number of slower waves with the single one in figure 2G. Some, however, may well be repetitive discharges from faster fibers as a result of relatively high stimulus strength. *B*. Potentials recorded from the posterior nerve during stimulation of the sciatic nerve by the method of figure 1B. As a result of the shorter conduction distance there is but a single major deflection. *C–D*. Potentials recorded from the medial nerve during stimulation of the 6th and 5th lumbar dorsal roots, respectively, showing the major deflections. Amplification approximately the same as for figure 2. *E*. Potentials recorded from the medial nerve during stimulation of the saphenous nerve showing but a single major deflection. Amplification approximately the same as for figure 3B. Time in milliseconds.

Medial nerve. In the case of the medial nerve, potentials were consistently recorded during stimulation of the 5th lumbar dorsal root, and, in three cases, of the 6th lumbar root as well. (In one case the femoral nerve was derived entirely from the 5th lumbar spinal nerve.) As in the case of the posterior nerve, the conduction distances were long, ranging from 14 to 19 centimeters. Again the temporal dispersion was great and the form of the potentials often complex. As a rule there were at least two, and sometimes three, major deflections (fig. 3C and

D). In all cases most of the first deflection represented fibers conducting at 30 to 60 meters a second, while in two cases there was a small initial component at 70 to 80 meters a second. The second major elevation was usually as large or larger than the first, and represented fibers conducting at rates of 20 to 30 meters a second. Succeeding this were either a single major deflection, or else variable smaller ones, the slowest of which conducted at nine or ten meters a second.

When the medial nerve was intact and the saphenous stimulated, no muscle contractions were ever seen, providing all other branches were cut (the branch of the femoral nerve to the pectineus may arise in conjunction with or directly from the saphenous). When the medial nerve was placed on electrodes and the saphenous stimulated, many more articular fibers were activated. Again, the potentials were larger, and, because the conduction distances were shorter, the major deflections were combined into one (fig. 3E). In one instance no potential was recorded during an experiment of this type, even though it had been successful during dorsal root stimulation. It was found that the medial nerve crossed over the saphenous and then pierced the adductor longus muscle and joined the obturator nerve. Subsequently it was found that the obturator sometimes gives a branch which joins the medial nerve, even when the latter is derived mainly from the saphenous.

DISCUSSION

The following conclusions may be drawn from these results.

1. Most, if not all, of these myelinated fibers are *A* fibers and are afferent in nature. If any are efferent, they would supply skeletal muscles in the neighborhood of the joint, and contractions of these muscles would then have been visible when the fibers were stimulated. This was never observed. If efferent fibers are present they must be nonmyelinated. It is quite likely that postganglionic vasomotor fibers may be included in this group.

2. It seems probable that conduction rates correspond to fiber diameters but there are two main reasons why a close fit cannot be made. The measurements of diameters were not made on the same nerves as those from which the potentials were recorded. Also, most of the determinations of conduction rates were made from records obtained during stimulation of dorsal roots. Since the articular fibers enter over several dorsal roots, there is no way of knowing the sizes of the fibers in a particular root. Fairly direct comparisons, therefore, can be made only between the largest fibers (about 16 microns in diameter) and the fastest rates (about 90 meters a second), and also between the smallest myelinated fibers (about two microns in diameter) and the slowest rates (about ten meters a second). According to Hursh (8) the ratio of diameter to conduction rate is a factor of six (using outside diameter, in contrast to the method of Gasser and Grundfest, 1939). This ratio is approximately realized in the instances cited here.

It is not unlikely that the ratio holds equally well for the other fibers and rates in which case fibers in the seven to ten micron group account for the first major deflections in the articular nerves. This group is more prominent in the posterior nerve and the corresponding potential is larger than any others. The succeeding major deflection in both nerves is probably obtained from fibers in the small, intermediate five to seven micron group and the two to five micron group. The latter is more prominent in the medial nerve and the corresponding potential is usually as large or larger than the first one.

In nearly all experiments, when dealing with threshold stimuli, there was a characteristic play of spikes, as described by Blair and Erlanger (3). The shock-spike distance varied from time to time even though the stimulus strength remained the same. Some of the variability may be attributed to utilization time, but in all cases the stimulus duration was much shorter than the shortest shock-spike distance observed under these conditions. Hence the variability is chiefly a result of changes in latency. In several experiments it was noticed that at threshold shocks the first spike to appear conducted more slowly than others which appeared with slightly stronger stimuli (fig. 2A and B). At the strengths used it is unlikely that this represents spread of excitation from the cathode, but rather that these were real differences in threshold, with a few fibers having a lower threshold than others with a slightly faster conduction rate.

3. The afferent fibers enter the spinal cord over several dorsal roots and the range of entry is surprisingly wide. The minimum is three dorsal roots, the 5th, 6th and 7th lumbar. In some cases the 1st sacral would be included as well, making four roots over which a total of approximately 315 myelinated fibers enter the spinal cord. Since the 6th lumbar dorsal root always contains fibers from the posterior nerve, and often from the medial as well, this root may well have the heaviest number of entering articular fibers.

In no instance were slow potentials of the *C* fiber type recorded, although they were searched for with very slow sweeps and maximum amplification. There are several possible explanations for this. One is that the potentials became so dispersed over the long conduction distances that they fell into the noise level of the amplifier (one to two microvolts). Others, however, have recorded *C* potentials after long conduction distances (11). It is also theoretically possible that the nonmyelinated fibers in these nerves are all autonomic, but it would be most surprising if this turned out to be the case. Probably the stimulus time used throughout these experiments was too short (one tenth to one millisecond), in which case either shocks of longer duration or greater strength or both should have been used. It is hoped that this point will be settled in future studies.

4. As yet, little can be said regarding the functions of these nerves and their endings. The nonmyelinated fibers form free endings in association with blood vessels and in the connective tissue of the capsule, particularly near Ruffini-type endings. The myelinated fibers form free and simple branched endings in connection with blood vessels, in the connective tissue of the capsule, and the larger ones form Ruffini-type endings in the posterior capsule at the zone of entrance of the posterior nerve (5). This is the portion of the capsule which is compressed during flexion of the knee joint. When the articular branch is severed from the tibial nerve, but left attached to the joint capsule, spontaneous potentials can be recorded (fig. 4). The causative stimuli have not been studied in detail, but it is possible that their origin is some stimulation of the Ruffini-type endings in the

posterior capsule. Whether this is a result of tension on the capsule by attached muscles remains to be determined. The potentials probably do not arise from the cut end of the nerve because difficulty of this sort was not encountered in other methods of recording. Furthermore, the potentials disappear when the nerve is cut or crushed between the electrodes and the joint. Most significant, however, is the fact that there is a definite increase in the frequency of these potentials when the posterior capsule is pressed lightly with a glass rod. Bursts of impulses were elicited by such a maneuver. Incidental observations indicate that these receptors are slowly adapting, but as yet the exact time of adaptation is unknown.

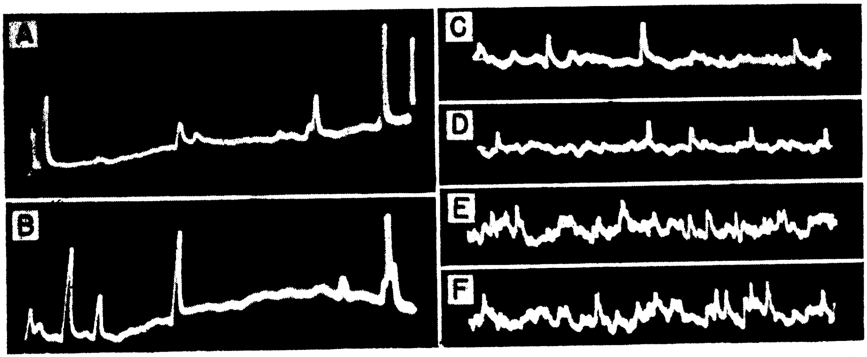

Fig. 4. *A–B*. Spontaneous potentials recorded from the posterior nerve without pressure applied to the capsule. *C–D*. Similar potentials recorded from a different posterior nerve. *E–F*. Same nerve as *C–D*, showing definite increase in frequency of potentials when light pressure is applied to the capsule.

SUMMARY

It has been determined that myelinated fibers in articular branches of the tibial, saphenous and obturator nerves are *A* fibers, that their conduction rates seem to correspond with the ranges of fiber diameters usually found in these nerves and that they are afferent in nature. They enter the spinal cord through the 5th, 6th and 7th lumbar dorsal roots, and sometimes the 1st sacral as well. There is no evidence that any of the fibers are motor, but the probability that many of the nonmyelinated fibers are vasomotor is not eliminated. Finally, it is presumed that the large spontaneous potentials which can be recorded arise from stimulation of Ruffini-type endings in the posterior capsule, because pressure in this region increases the frequency of the potentials.

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REFERENCES

1. Adrian, E. D. Brain **66:** 289, 1943.
2. Barnes, T. C. This Journal **100:** 481, 1932.

3. Blair, E. A. and J. Erlanger. This Journal **106:** 524, 1933.
4. Comroe, J. H. and C. F. Schmidt. This Journal **138:** 536, 1943.
5. Gardner, E. D. J. Comp. Neurol. **80:** 11, 1944.
6. Gasser, H. S. and H. Grundfest. This Journal **127:** 393, 1939.
7. Harrison, T. R., W. G. Harrison, J. A. Calhoun and J. P. March. Arch. Int. Med. **50:** 690, 1932.
8. Hursh, J. B. This Journal **127:** 131, 1939.
9. Leriche, R. Presse méd. **38:** (1): 417, 1930.
10. Raszeja, F. and J. Billewicz-Stankiewicz. Abs. compt. rend. soc. biol. **115:** 1267, 1934.
11. Sheehan, D. and A. S. Marrazzi. J. Neurophysiol. **4:** 68, 1941.
12. Stopford, J. S. B. J. Anat. **50:** 1, 1921.

URINARY EXCRETION OF ASCORBIC ACID BY GUINEA PIGS WITH HEALING SKIN WOUNDS

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The necessity of ascorbic acid for the synthesis of collagen is now generally recognized (1, 2). Its rôle in the healing of wounds is presumably related chiefly to this function (3–6), though whether the vitamin serves as a building stone or as a catalyst in collagen formation has not yet been demonstrated. Convincing evidence has been presented of greater retention of ascorbic acid following injury as will be noted in the following:

Lauber and Rosenfeld (7) found that ascorbic acid tends to concentrate in and around wounded areas. Bartlett, Jones and Ryan (8) confirmed these results and showed in addition that the content of the vitamin in the wounded area varies with the ascorbic acid intake. They found as much as 6.53 mgm./100 grams in scar tissue of guinea pigs on a preoperative and postoperative diet high in vitamin C.

Andrae and Browne (9, 10) have recently reported results of a study of ascorbic acid metabolism after trauma in man and found that all of the injured subjects showed a low urinary output with large doses of ascorbic acid and that the retention was greater after burns than after fractures. Following the injury the level in the blood fell markedly and failed to rise after 15 to 20 days on the high intake suggesting destruction or utilization of the vitamin. A high ascorbic acid retention lasting only one to two days similar to that in normal individuals was observed several months after recovery from the injury. The evidence was considered inconclusive as to whether the ascorbic acid is destroyed or is incorporated in the newly-formed tissues. The fact that the retention was greatest immediately after the injury and became less so later when the tissue anabolism was increased suggested that the vitamin was not being used up in the formation of new tissues.

Levenson *et al.* (11) determined the plasma level and urinary excretion of ascorbic acid in six patients with severe surgical conditions to whom ascorbic acid was administered intravenously. They found abnormally small amounts in both blood and urine during the period of acute illness. Their findings were interpreted as affording no evidence as to whether or not the vitamin serves a useful purpose during its disappearance.

The need of further study of the rôle of ascorbic acid in wound healing is evident, particularly with the objective of determining whether any of the vitamin is utilized in anabolic processes.

EXPERIMENTAL METHODS

Adult male guinea pigs of an inbred strain[1] which had been kept saturated with ascorbic acid since shortly after weaning were used as test animals. Animals were selected which had shown a moderately steady excretion over a long period. Those used in the first test were approximately nine months old and ranged in weight from 710 to 950 grams with an average weight of 820 grams, and those in the second test, which was conducted several months later, varied from 750 to 970 grams with an average weight of 880 grams. The animals were placed in metabolism cages and were fed a scorbutogenic pelleted stock diet (commercial) supplemented with one per cent of powdered yeast. Ascorbic acid was supplied by daily intraperitoneal injections of 5 mgm. for each 100 grams of body weight. The vitamin was dissolved (15 mgm/ml.) in 0.5 per cent solution of sodium bicarbonate to partially neutralize the acidity. Each animal received the same (as the initial) dosage throughout the experiment.

Ten grams of crystalline metaphosphoric acid was placed each day in each of the bottles used for collecting the urine. This amount was usually sufficient to afford protection to the ascorbic acid excreted during a 24-hour period in a room maintained at 76 to 80°F. Some difference was observed in the quantity of metaphosphoric acid required by the urines of different animals and was attributed in part to differences in the volume of fluid collected in the bottles. In preliminary tests it was observed that some of the animals spilled water as they drank, thus diluting the metaphosphoric acid considerably. By providing drinking tubes with very small openings for animals inclined to spill the water some control was obtained of the volume of fluid collected.

Assays of the ascorbic acid excreted were made daily by both the indophenol method and the osazone method of Roe and Kuether (12) to determine the reduced and total ascorbic acid, respectively. The excretion was determined during the 9-day period previous to wounding, throughout the period of active healing (11 days in the first test and nine days in the second) and for nine days following complete healing of the wounds with the exception that osazone determinations were made for only seven days during the recovery period in the first experiment. The wounding[2] was effected by cutting through the skin down the center of the back for a distance of 10 cm. The cut edges were fastened together with skin clips which were removed on the ninth day after wounding, at which time healing was well advanced. Precautions were taken to keep the animals well protected from draughts and also to maintain their room at slightly higher temperature than usual during the first 24-hour period after wounding. The food and water intake was slightly less than usual during this period and became normal by the second day. None of the pigs showed delayed healing due to infection.

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[1] Animals from family No. 13 were used. This strain was developed by the Genetics Section of the Bureau of Animal Industry of the U. S. Dept. of Agriculture. Later, the line was continued by the Genetics Section of the National Cancer Institute, from which the animals here used were obtained.

[2] The writer is indebted to Dr. Thelma B. Dunn of the National Cancer Institute for the surgical operations.

EXPERIMENTAL OBSERVATIONS

After preliminary experiments in which the necessary methods were developed, two tests each with eight animals were conducted, the results of which corresponded fairly well within the limits of error of the method. The results of the two tests with both methods are shown in figures 1 and 2. The data indicate a real change in excretion during the healing period. The rather wide deviations occurring throughout the three experimental periods are caused in part by differences in weight and dosage of the animals and in part by individual differences[3] in the average daily excretion. The horizontal lines at the left in the figures represent the average excretion values during the prewounding period and those at the right the average values during the period after healing was apparently complete. The differencs in results by the two methods of assay were not great.

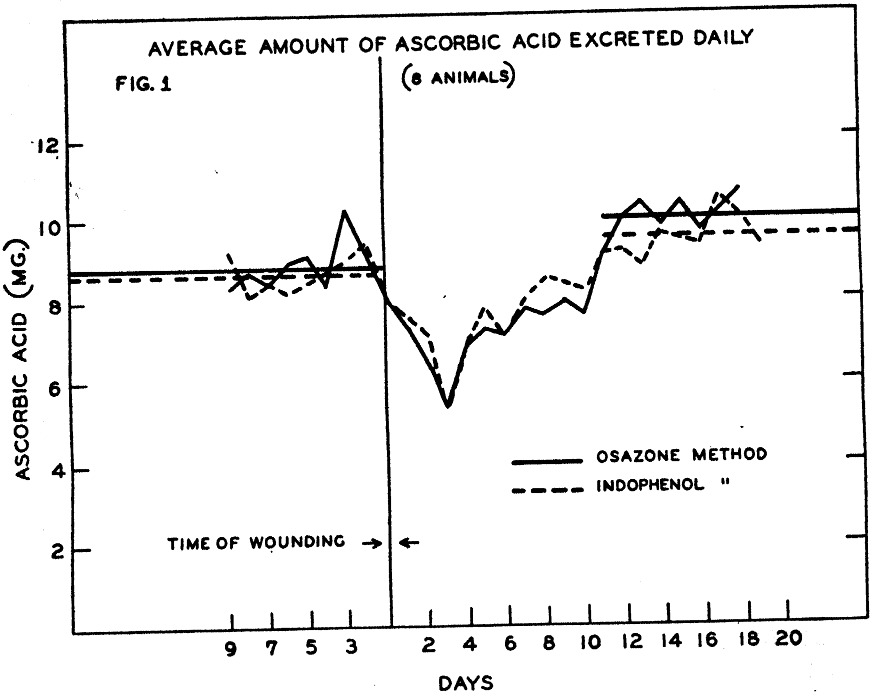

Fig. 1

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[3] In other studies (13) it has been found that guinea pigs excrete (excess) intraperitoneally injected ascorbic acid into the digestive tract where it is fairly rapidly destroyed. There are individual variations in the rapidity of this process, the differences probably resulting from variations both in the rate of excretion into the digestive tract and in its subsequent destruction. This variability is reflected in the blood concentration and consequently would presumably influence the amount removed by the kidneys.

The generally higher values obtained with the osazone method, shown particularly in figure 2, are presumably due to the presence of small amounts of dehydroascorbic acid. The low value obtained with the osazone method on the fourth day previous to wounding in the second test (fig. 2) is probably a result of an analytical error, the cause for which is not known.

After the wounding there was a drop in the excretion values in both experiments. The most striking feature of the results is the definite fall by the third day with the values remaining low for several days. In both tests all of the animals but one showed this depression in excretion after wounding, with a return to normal values during the recovery period. The quantitative data for the total excretion values per animal during the three experimental periods are shown in tables 1 and 2. The general level of excretion during the prewounding period in the first test (table 1) was somewhat lower than is generally expected under these experimental conditions. The return to normal excretion values occurred by the eleventh or twelfth days in the first test and by the ninth day in the second. This difference in time was correlated with an apparent difference in the rate of wound healing. The animals in the second test, which required the shorter time, had in most cases, a more rapid rate of healing. During the period of active healing there was a decrease in the total amount of ascorbic acid normally excreted of 16.5 mgm. or 18.6 per cent per individual animal in the first test and in the second test a decrease of 12.4 mgm. or 12.9 per cent.

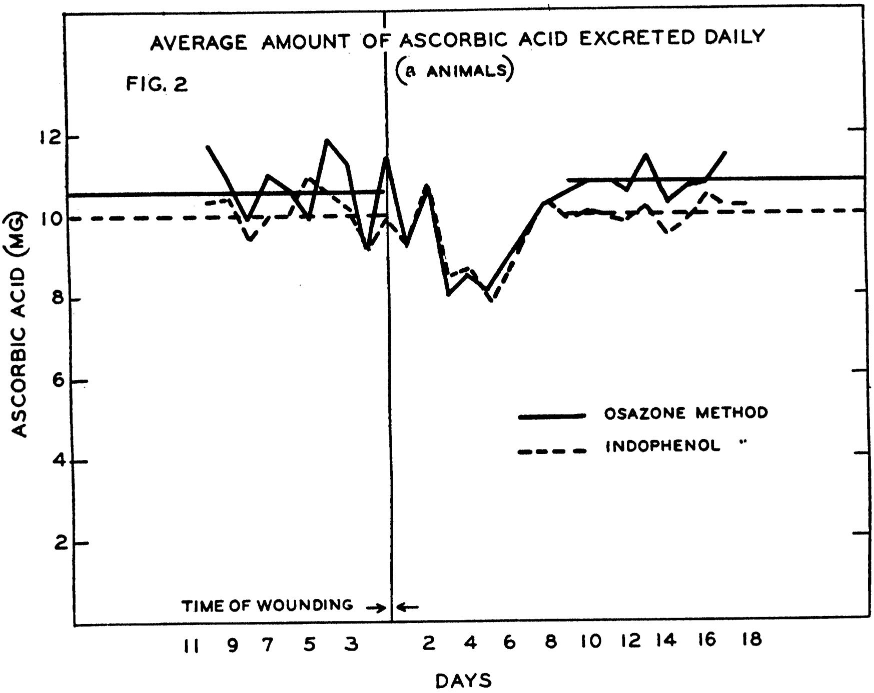

FIG. 2

The averages for the standard deviations in excretion per individual were slightly higher during the healing than during the prewounding period. The average values for the prewounding, healing and recovery periods, respectively, were 0.91, 1.01 and 0.74 mgm. in the first test and 0.85, 1.00 and 0.98 mgm. in the second (tables 3 and 4). If a four consecutive-day interval in the middle of each

TABLE 1. TOTAL ASCORBIC ACID EXCRETED PER ANIMAL (EXPT. 1) DURING 7 TO 10-DAY PREWOUNDING, HEALING AND RECOVERY PERIODS

(Osazone Method)

GUINEA PIG NUMBER	PREWOUNDING 9 DAYS	HEALING 10 DAYS	RECOVERY 7 DAYS
	mgm.	*mgm.*	*mgm.*
24	78.0	52.1	54.6
26	67.5	49.8	66.6
28	74.3	62.0	70.1
29	58.6	52.4	61.5
31	91.0	110.4	83.6
32	119.5	112.1	92.8
33	63.5	60.2	55.4
35	84.9	77.0	74.5
Total..............	637.3	576.0	559.1
	708.1*		798.7†
		−15.3 mgm.	+11.3 mgm.
		−18.6 %	+12.8 %

* Excretion for 10 days (calculated).
† Excretion for 10 days (calculated).

TABLE 2. TOTAL ASCORBIC ACID (MGM.) EXCRETED PER ANIMAL (EXPT. 2) DURING THE 9-DAY PREWOUNDING, HEALING AND RECOVERY PERIODS

(Osazone Method)

GUINEA PIG NUMBER	PREWOUNDING	HEALING	RECOVERY
25	82.9	86.0	83.4
26	95.4	69.0	82.4
27	81.2	67.1	81.5
28	112.4	103.0	132.0
29	92.2	77.9	95.9
32	118.8	107.8	124.8
33	86.3	74.5	91.9
35	96.5	81.4	88.6
Total..............	765.7	666.7	780.5
		−12.9 %	+1.9 %
		−12.4 mgm.*	

* Per animal.

TABLE 3. AVERAGE AMOUNTS OF ASCORBIC ACID EXCRETED DAILY DURING THE PREWOUNDING, HEALING AND RECOVERY PERIODS (EXPT. 1)

(Osazone Method)

PREWOUNDING		HEALING			RECOVERY		
Days before wounding	Mean	Days after wounding	Mean		Days after wounding	Mean	
	mgm.		mgm.			mgm.	
1	8.10 ±0.70	1	7.41 ±0.99		11	9.06 ±0.95	
2	9.26 ±0.82	2	6.51 ±1.02		12	9.96 ±0.68	
3	10.32 ±0.91	3	5.49 ±1.48	*	13	10.38 ±0.32	†
4	8.42 ±1.07	4	7.40 ±1.12		14	9.76 ±0.81	
5	9.13 ±0.96	5	7.70 ±1.13		15	10.39 ±0.76	
6	8.96 ±0.66	6	7.17 ±1.02		16	9.68 ±0.75	
7	8.42 ±1.08	7	7.80 ±0.55		17		
8	8.77 ±0.97	8	7.65 ±1.14		18	10.65 ±0.94	
9	8.31 ±0.98	9	7.99 ±0.78				
		10	7.68 ±0.90				
Mean.....	8.85 ±0.91		7.28 ±1.01			9.98 ±0.74	

Mean for comparison periods: prewounding, 9.21 ±0.90; healing, 6.94 ±1.19; recovery, 10.05 ±0.66.

* −24.6%.

† +9.1% comparison period.

TABLE 4. AVERAGE AMOUNTS OF ASCORBIC ACID EXCRETED PER ANIMAL DAILY DURING THE PREWOUNDING, HEALING AND RECOVERY PERIODS (EXPT. 2)

(Osazone Method)

PREWOUNDING		HEALING			RECOVERY		
Days before wounding	Mean	Days after wounding	Mean		Days after wounding	Mean	
	mgm.		mgm.			mgm.	
1	11.33 ±0.65	1	9.13 ±0.95		10	10.76 ±0.54	
2	9.17 ±0.77	2	10.61 ±0.67		11	10.81 ±1.20	
3	11.29 ±0.89	3	7.99 ±0.93	*	12	10.48 ±1.13	†
4	11.83 ±0.66	4	8.52 ±0.98		13	11.38 ±1.19	
5	9.79 ±0.35	5	8.10 ±0.85		14	10.18 ±1.00	
6	10.62 ±0.82	6	8.78 ±0.88		15	10.65 ±1.13	
7	11.00 ±1.42	7	9.46 ±1.01		16	10.80 ±1.06	
8	9.82 ±1.03	8	10.23 ±1.63		17	11.45 ±0.79	
9	10.85 ±1.05	9	10.51 ±1.08		18	11.04 ±0.75	
Mean.....	10.63 ±0.85		9.26 ±1.00			10.84 ±0.98	

Mean for comparison periods: prewounding, 10.88 ±0.68; healing, 8.35 ±0.91; recovery, 10.67±1.11.

* −23.2%.

† +1.9% comparison period.

of the three experimental periods is selected for purposes of comparison, it is found that the average excretion during the healing period was 24.6 per cent lower than that of the prewounding period in the first experiment and 23.2 per cent lower in the second. During the recovery period there was a return to values slightly higher (9.1 per cent and 1.9 per cent in the first and second tests, respectively) than in the comparable prewounding period.

Because of the necessity of collecting the urine in metaphosphoric acid there was no opportunity to follow any possible changes in the urinary pH values. The pH values of the urines of these animals determined shortly before starting the present experiments varied from 7.95 to 8.50. Because of the alkaline reaction some oxidation of the vitamin probably occurs during the period in which the urine is retained in the bladder (14), even though the oxygen tension is doubtless low. At these high alkalinities destruction of dehydroascorbic acid doubtless occurs rapidly, which would account for the low values found for dehydroascorbic acid (difference between total and reduced).

DISCUSSION

The feature of special interest in the results of these experiments is that they show that the period of diminished excretion of the vitamin in the urine coincides roughly with the known period of collagan formation.

Hunt (5) showed that with a sufficient supply of ascorbic acid a mature vascular scar is built up in wounded guinea pigs within 14 days. Hartzell and Stone (6) also found that the strength of healing wounds in the abdominal walls of guinea pigs adequately supplied with ascorbic acid increased sharply after six days and reached the strength of the intact abdominal wall by the 14th day. Mazoue (15) had previously studied the rate of production of collagen fibers around a mass of Kieselguhr injected intraperiteoneally into guinea pigs and demonstrated a direct relation between the dosage of ascorbic acid and the time of the first appearance of fibroblasts and fibers. The time of greatest apparent cellular development occurred from around the fourth to the sixth days. The types of cells invading and developing in the wounded area varied at different stages, the polymorphonuclears, which were most conspicuous on the first and second days were less numerous by the fourth day. Cells of connective tissue type were noticeable on the second day and fibrils of the fibroglia fibril type had by this time formed a net surrounding the mass of Kieselguhr. Connective tissue cells predominated by the fourth day and showed a high degree of activity. At this time fibrils of precollagen were very numerous and more collagen fibers could also be seen. By the sixth day the fibers were much more numerous, they were thickening and uniting into bundles, encapsulating the mass of Kieselguhr. By the eighth and tenth days the mass was completely encapsulated and was traversed throughout by fibers. By this stage all of the precollagen fibers had been changed to collagen. On the 13th day the process of fibrosis was observed to be continuing until it formed a complete layer about the entire mass of Kieselguhr.

Although there is a rough correlation between the periods of increased ascorbic acid metabolism and of collagen production during wound healing, actually there appears to be a slight time difference in the maxima for the two processes.

The period of maximal disappearance of the vitamin from the urine in the present tests extended from the second to the sixth day, thus preceding slightly the known period of most active fiber production. Hence it would appear to coincide somewhat more closely with the period of maximum connective tissue cell activity.

The amounts of the vitamin which were found in scar tissue of guinea pigs by Bartlett, Jones, and Ryan (8) namely, 6.53 mgm. per 100 grams of scar tissue, could not account for the urinary output decrements found during wound healing (15.3 and 12.4 mgm.) in the present tests unless some utilization or destruction of the vitamin also occurs.

There is a discrepancy between the present results and those of Andrae and Browne (9) as to the time at which greatest retention of the vitamin occurs. They found the greatest retention immediately after injury whereas in the present tests it occurred three to six days later. The discrepancy may be explained by the difference in type of subjects, but it seems probable that it may be found, at least in part, in a different direction. In their subjects with accidental injuries there probably was a much greater relative amount of actual tissue destruction than in the clean-cut skin wounds of the present tests. Presumably, the greater the amount of cellular damage, the greater may be the actual destruction of the vitamin, a condition which may account for the greater retention immediately after injury. Later, as the cellular elements migrate into the wounded area and become active, they, or at least certain types of these cells, may (1) very actively absorb the vitamin and (2) may possibly utilize it in anabolic processes associated with healing. Evidence lending indirect support to these suggestions is to be found in the ratio of dehydro to reduced ascorbic acid in the urine. Muntoni (16) reported that after surgical operations the ratio of dehydro to reduced ascorbic acid increased. Andrae and Browne (9, 10) found also that immediately after injury the ratio increased but that during convalescence the ratio was the same as in the normal condition. The fairly large amounts of dehydro ascorbic acid formed immediately after injury may be interpreted as indicating destruction of the vitamin catabolically and the small amount or none formed during convalescence as indicating little or no destruction. The formation of little or no dehydroascorbic acid as revealed in their studies during healing suggests the possibility that the retention characteristic of this period may be a consequence of anabolic processes. In the present tests there is some indication that less rather than more dehydro ascorbic acid is formed in the healing period than in the prewounding and recovery periods. This is shown particularly in figure 2 in which the curves obtained by the two methods are divergent during the prewounding and recovery periods but follow each other rather closely during the healing period. Although it is possible that the apparent decrease in dehydroascorbic acid during healing is fortuitous, the suggestion is nevertheless advanced that since a more rapid catabolism of protein is a characteristic accompaniment of injury (17), presumably the urine at this time may have a somewhat lower pH and as a consequence less of the vitamin is changed into the dehydroform.

SUMMARY

Experiments were conducted to determine if ascorbic acid is used up in the healing of skin wounds in guinea pigs. Incisions 10 cm. long were made down the centers of the backs of adult animals. Daily intraperitoneal injections of the same amounts of ascorbic acid were continued through a nine-day pre-wounding period, through the period of active healing (9 to 11 days), and for 7 to 9 days following closure of the wounds. Daily determinations of the ascorbic acid excreted in the urine were made by both the osazone and indophenol titration methods.

In each of the two tests conducted with eight animals each, it was found that a depression in the urinary excretion of ascorbic acid occurred during the period of most active healing by both methods of assay following which there was a return to the prewounding values. The greatest depression occurred from the second to the sixth days. During the nine-day period of active healing there was a decrease of 15.3 mgm. or 18.6 per cent of the total amount normally excreted in one test and of 12.4 mgm. or 12.9 per cent in the other. The difference between the output of a four consecutive-day period at the time of lowest excretion with a comparable prewounding period was 25 and 23 per cent, respectively, in the two experiments.

The results suggest that less dehydroascorbic acid is produced in the guinea pig during the period of active healing than in the prewounding and recovery periods.

The period of greatest metabolism of ascorbic acid preceded slightly the known period of most active fiber formation, hence it appears to parallel somewhat more closely the known period of maximal connective tissue cell activity.

REFERENCES

1. Wolbach, S. B., New Engl. J. Med. **215**: 1158, 1936.
2. Mazoue, H. Compt. rend. soc. biol. **126**: 991, 1937.
3. Crandon, J. H. and C. C. Lund. New Engl. J. Med. **222**: 748, 1940.
4. Crandon, J. H., C. C. Lund and D. B. Dill. New Engl. J. Med. **223**: 353, 1940.
5. Hunt, A. H. Brit. J. Surg. **28**: 436, 1941.
6. Hartzell, J. B. and W. E. Stone. Surg. Gynecol. Obstet. **75**: 1, 1942.
7. Lauber, H. J. and W. Rosenfeld. Klin. Wochschr. **17**: 1587, 1938.
8. Bartlett, M. K., C. M. Jones and A. E. Ryan. Ann. Surg. **111**: 1, 1940.
9. Proc. Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing. 9th Meeting pp. 34–44, 1945; 10th Meeting pp. 307–9, 1945.
10. Andrae, W. A. and J. S. L. Browne. Can. Med. Assoc. J. **55**: 425, 1946.
11. Levenson, S. M., R. Green, T. H. L. Taylor, P. Robinson, R. C. Page, R. E. Johnson and C. C. Lund. Ann. Surg. **124**: 840, 1946.
12. Roe, J. H., and C. A. Kuether. J. Biol. Chem. **147**: 399, 1943.
13. Reid, M. E. Federation Proc. **6**: 418, 1947.
14. Koops, H. Med. Welt. **16**: 1115, 1942. Abt. Ber. ges. Physiol. **132**: 535, 1943.
15. Mazoue, H. Arch. Anat. Micr. **33**: 129, 1937; **35**: 91, 1939.
16. Muntoni, E. Sperimentale **96**: 234, 1942.
17. Proc. Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing. 8th and 9th meetings, 1944, 1945.

ROENTGENOGRAPHIC STUDY OF MOTILITY OF GASTRO-INTESTINAL TRACT OF THE GUINEA PIG

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Investigations dealing with the rate of movement of the contents through the different regions of the gastro-intestinal tract have been made with several types of laboratory animals. Magnus (1) studied the progress of digestion in cats and dogs starved for 24 hours using the roentgenographic technic developed by Cannon (2, 3). He fed cats each five grams of bismuth subnitrate in 25 ml. of potato brei, and dogs were given 7.5 grams of bismuth subnitrate mixed with 50 ml. of broken dog biscuit moistened with water. In similar studies with rats Menville, Ané and Blackberg (4) fasted the animals for 48 hours, withheld water 24 hours before feeding a meal composed of 10 grams of $BaSO_4$ and 10 ml. of buttermilk. Gershon-Cohen, Shay and Fels (5) conducted similar investigations on rats and found considerably more rapid movement of the contents of the digestive tract, which may possibly be explained in part by differences in experimental procedure. Their animals were deprived of food and water only 18 hours prior to the roentgenographical studies. The test meal consisted of three ml. of a water suspension of barium sulphate put directly into the stomach by a stomach tube. The results of these various studies together with one on human subjects (6) are summarized in table 1.

No reports have been found of investigations dealing with the rate of movement of the contents of the gastro-intestinal tract of guinea pigs. For another study, initiated to investigate the disappearance from guinea pigs of a relatively large proportion of the ascorbic acid injected intraperitoneally, such information was found to be essential.

Twenty-seven roentgenographic studies using tests meals of a water-barium sulphate mixture were accordingly made on 17 adult animals of an inbred strain maintained on a commercial pelleted diet supplemented with one per cent of dried powdered yeast. Ascorbic acid was injected intraperitoneally (daily) in the proportion of five mgm. per 100 gram of body weight. A 0.5 per cent solution of sodium bicarbonate was used as a solvent for the vitamin (15 mgm./ml.). The animals were kept on this regime for four weeks or longer before making the studies. In some of the tests the food was removed from the cages the night before the barium was administered; in others it was removed at 4 A.M. the day of the tests. At 8 A.M. five ml. of a creamy mixture of 10 grams of barium sulphate in 20 ml. of water was introduced into the stomach by stomach tube. Roentgenograms[1] were taken immediately and after 1, 2, 3, 4, 6, 8, 10 and 24 hours. After the fourth hour the food was replaced in the cages.

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[1] $\frac{1}{10}$ sec., 30 milliamps, 62 kilovolts, 30″ distance.

TABLE 1. Rate of movement of contents through gastrointestinal tract in different animals

ANIMAL	STOMACH: EMPTYING TIME	SMALL INTESTINE			CECUM		LARGE INTESTINE: EMPTYING TIME	INVESTIGATORS
		First appearance	Maximum filling	Emptying time	First appearance	Emptying time		
	hr.	*min.*	*hr.*	*hr.*	*hr.*	*hr.*	*hr.*	
Cat	3	15	2	8–9	2	—	—	Magnus (1)
Dog	2.5	15	2	6–7	about 2	—	—	Magnus (1)
Rat	6 hrs. 14 min.	—	—	10 hrs. 26 min.	3 hrs. 37 min.	—	65	Menville, Ané and Blackberg (4)
Rat	1–3	—	—	—	3–5	—	24	Gershon-Cohen, Shay & Fels (5)
Man	4.6	25	—	6.5	3.3	17.3	25.5	Zehbe (6)
Guinea pig	1+	less than 1 hr. Exact time not det'd	1	2	less than 1	8–10	24– to 24+	Reid and White (this paper)

TABLE 2. Location of barium meal in the digestive tract of guinea pigs at successive periods. Twenty-seven studies on 17 animals

ORGANS	HOURS AFTER ADMINISTRATION OF BARIUM SULPHATE							
	1	2	3	4	6	8	10	24
Stomach	½ to ¾ out	Almost out	—	—	—	—	—	—
Small intestine	throughout entire length	Almost out	all out except trace	—	—	—	—	—
Cecum	entering in 6 of 27	½ to ⅘ of total in	½ to ¾ in	½ to ¾ in	½ to ⅕ in	⅓ to trace in	all out or trace	—
Large intestine	—	Entering	small amount to ⅓ in	¼ to ½ in	½ to ⅘ in	feces escaping in 5	feces escaping in 9	all out in 16. Small amount in 6. Trace in 5

OBSERVATIONS

At the end of the first hour from a third to a half of the barium was still in the stomach, the remainder being distributed throughout the entire length of the small intestine. By the end of two hours most of the barium had passed into the

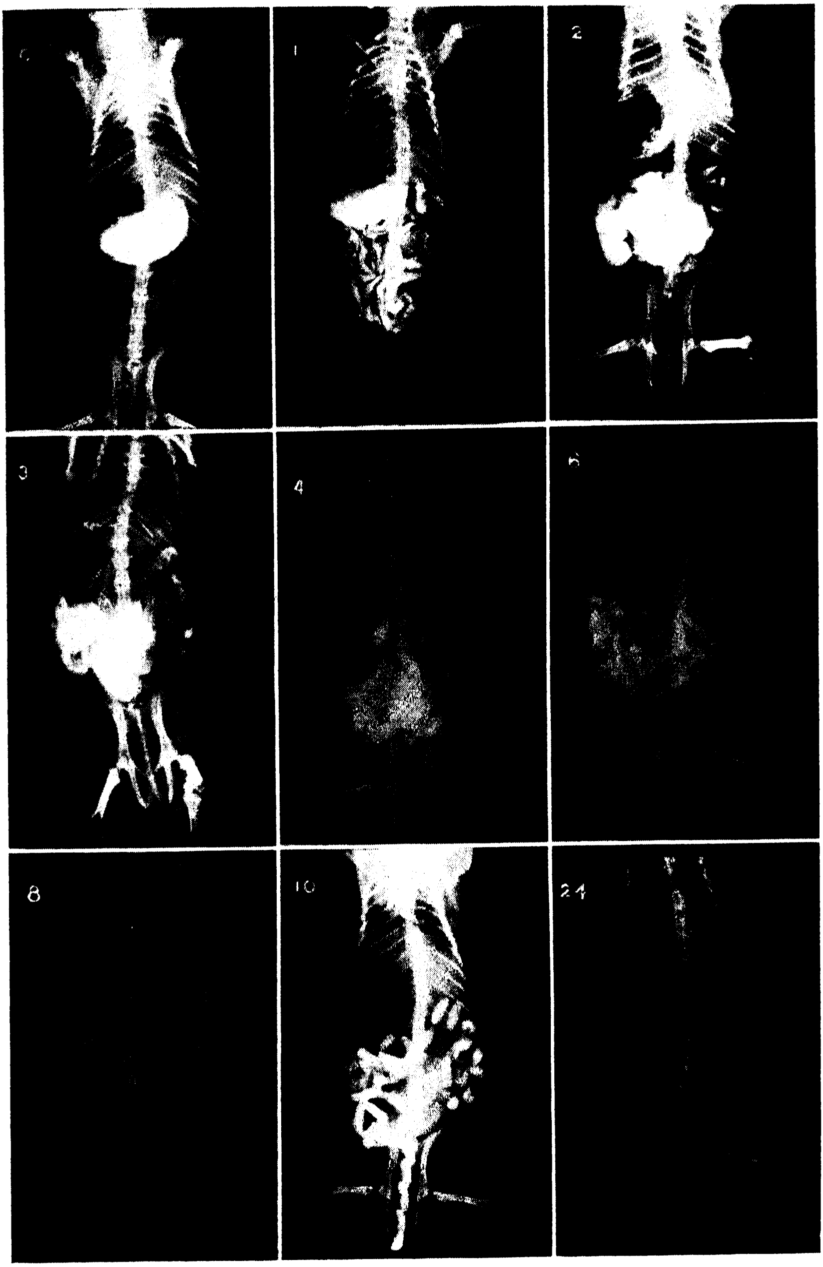

FIG. 1. LOCATION OF BARIUM AFTER DIFFERENT INTERVALS OF TIME. Figures represent number of hours after administration. The photographs do not represent the gastro-intestinal tract in any one individual but rather illustrate the average position of the barium for each time interval as near as the average could be determined.

cecum and in most of the animals it could be detected entering the large intestine. Only small amounts were left in the stomach and small intestine in most of the animals. Some of the barium remained in the cecum at least five hours and in many cases longer. By the eighth hour it appeared to be almost all out of the cecum. After eight hours, feces containing barium were escaping in five of the 27 animals. After 24 hours the barium had all been excreted in 16 of the 27 animals, there was a trace left in the large intestine in five of them and small amounts in six. No definite differences were observed in the rate of movement of the contents of the gastro-intestinal tract between the animals deprived of food over night and those without it for only four hours previous to administering the barium.

The results of the above studies are summarized in table 2 and typical roentgenograms are shown in figure 1.

DISCUSSION

The most interesting feature of these results is the rapid progress of the barium through the stomach and small intestine of the guinea pig and the marked retardation in the cecum. The passage through the stomach is faster than that observed in the cat (1), dog (1) and man (6) and the progress through the small intestine is much more rapid than in these other animals. In general, the movement is more like that found in the rat (5), though there is a definite difference in the time required for passage through the small intestine. The first appearance of the barium was observed in the cecum of the rat in from three to five hours, whereas in the guinea pig it entered within the first hour and by the end of the second hour all but a small amount had passed in.

As to whether barium may induce more or less rapid propulsion than would normal food stuffs no definite answer can be given at the present time. Judging by the results obtained under similar conditions with human subjects, however, it may be assumed that the movement is practically normal.

The chief significance in the results is to be found in the influence of time as a factor in the absorption of special types of nutrients. Since there is considerable evidence that much if not most of the absorption of vitamin C, for example, occurs through the walls of the small intestine and possibly to some extent in the stomach, the short period during which the contents are exposed to these absorbing surfaces becomes of critical importance. From these results and the data presented in a previous publication (7), it appears probable that if the daily quota of this vitamin is given in one dose or in a form not readily available for absorption there may be considerable loss if the substance is propelled forward out of the small intestine before absorption is complete.

SUMMARY

The roentgenological-barium sulfate technic commonly employed in clinical determinations of gastro-intestinal propulsion rate was applied in guinea pigs to a study of the rate of movement of contents through the different regions of the gastro-intestinal tract.

The chief feature of interest in the results is the rapid movement through the stomach and small intestine and the marked retardation in the cecum. Within

a period of two hours most of the barium had moved out of the stomach and small intestine. After 24 hours all of the barium had been eliminated in 16 of the 27 animals, traces only were left in five animals, and small amounts in six.

The significance of the results is discussed in connection with possible failure in the complete absorption of special types of nutrients such as some of the vitamins, particularly when a 24-hour quota in high dosage is administered at one time, or in a form not readily accessible for absorption.

REFERENCES

1. Magnus, R. Arch. ges. Physiol. **122:** 210, 1908.
2. Cannon, W. B. This Journal **1:** 359, 1898.
3. Cannon, W. B. The Mechanical Factors of Digestion. International Medical Monographs, London: Edward Arnold & Co., 1911.
4. Menville, L. J., J. N. Ané and S. N. Blackberg. Proc. Soc. Exp. Biol. Med. **26:** 758 1929.
5. Gershon-Cohen, J., H. Shay and S. S. Fels. Am. J. Roentgenology **46:** 876, 1941.
6. Zehbe, N. Therap. Monatsh. **27:** 406, 1913.
7. Reid, M. E. Federation Proc. **6** (1): 418, 1947.

EFFECT OF FEEDING LIVER ON THE RATE OF REGENERATION OF THE LIVER IN PARTIALLY HEPATECTOMIZED RATS

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This study was undertaken to ascertain whether or not the feeding of liver to partially hepatectomized rats augments the rate of regeneration of the liver.

This question was raised because we were interested in the more basic question of the nature of the stimulus or stimuli responsible for the remarkable regenerative ability of the liver. The stimulus which is responsible for the regeneration of the liver may be extrinsic or intrinsic in origin. If the stimulus is intrinsic and is not mechanical, then it should be present either in the nonregenerating liver or in the regenerating liver. If such a factor be present in the liver in either state, it should be detectable by feeding whole liver in large amounts or by injecting appropriately prepared extracts or concentrates of the liver into partially hepatectomized rats. If a favorable effect occurred on feeding liver, the possibility that the effect might be due to the quality of liver protein (1) would have to be considered. It was thought that the best approach to the answer of the basic question was to ascertain the effect of feeding a diet of liver on the rate of regeneration of the liver as compared to the feeding of a stock diet.

We have been unable to find any literature dealing directly with this question. McJunkin and Breuhaus (2) observed a great increase in mitosis in the hepatic remnant of partially hepatectomized rats which had received injection of 'macerated liver substance'. Marshak and Walker (3) injected various liver fractions into partially hepatectomized rats and observed an increase in mitosis when fat-free chromatin was injected. They found in addition that chromatin labeled with P32 was rapidly assimilated into the intracellular substance of the regenerating liver. This work suggests that the liver may contain some factor which stimulates regeneration.

EXPERIMENTAL

Technique of removing the liver. At operation, the method of Anderson and Higgins (5) and Brues, Drury, and Brues (4) was used. It should be mentioned that the portal vein leading to the lobes to be removed was clamped for three minutes to allow for partial exsanguination of the lobes before their pedicle was tied. The portion removed was allowed to drain of blood and was then weighed to the nearest five milligrams.

At 'necropsy', as in the 32 rats cited below, the rat was anesthetized on the tenth to eleventh day (average, 10.7 days), the abdomen was opened, the portal vein and hepatic artery were clamped, and then after two or three minutes the vena cava was clamped and the liver removed. The liver was allowed to drain of blood, weighed wet, and then dried to constant weight in a constant temperature oven at 100° C., it having been found that further desiccation in a desiccator did not decrease the weight.

Amount of liver removed. We desired to remove approximately 70 per cent of the liver as others have done. In this connection Brues, Drury and Brues (4) found that the left lateral and the median lobes of the rat's liver constituted 68.5 per cent of the total weight of the liver. Or, in 13 rats from their colony they found that if the weight of these lobes was multiplied by the factor 1.46, the total weight of the liver could be approximated. This factor of 1.46 had a standard deviation of only ±0.03 for the series of 13 rats they used. This, of course, served as a basis for the amount of liver regenerated after partial hepatectomy. They found that this method had a smaller coefficient of variability than when the formula for body-weight to liver-weight relationships was used (5).

To establish for our colony of rats the validity of the method of Brues *et al.* (4) for estimating the total weight of the liver at the time of operation, we removed the left lateral and median lobes of the liver and weighed them; then we removed and weighed the remainder of the liver (left central, right, and caudate lobes).

TABLE 1

RATS	AVR. BODY WT. AT OP. (GRAMS)	AVR. WT. OF MED. AND LEFT-LAT. LOBES REMOVED AT OP.	AVR. WT. OF RIGHT AND CAUDAL LOBES REMAINING	AVR. WT. OF TOTAL LIVER
32	180.4 ± 4.3[1]	1.798 gm.	0.824 gm.	2.628 gm.

$$\text{Thus, the ratio of } \frac{\text{med. and left lat.}}{\text{Total}} = \frac{1.798}{2.623} = 68.56\% \pm 0.27 \text{ (P.E.)}^1$$

Range of percent of liver removed = 64.0% to 71.3%.

[1] Probable error of the mean.

Thirty-two albino rats, which weighed from 150 to 300 grams and which were approximately equally distributed between the sexes, were used.

The averaged results are shown in table 1. The value of 68.56 per cent ± 0.027 (P.E. of the mean) checks the percentages of Brues very precisely, the factor being 1.46 ± 0.047 (standard deviation).

The effect of feeding a diet of liver on the rate of regeneration. Prior to operation the rats were kept on the stock diet used in our laboratory and water. The diet had the following composition: P = 22%, CHO = 45%, F = 4.0%, and fiber = 5%, and was adequate to support normal group and reproduction. The composition of the pigs' liver was approximately: P = 20%, CHO = 1.0%, and F = 4.8%. The 'control group' was given the diet and water after operation. The 'liver-fed group' was given only ground pigs' liver which had been brought to a boil to stop autolysis and to retard putrifaction. The food and water were given *ad libitum*, and the rats were kept in individual cages. No pre- or post-operative withdrawal of food was practiced; neither was saline or glucose given post-operatively. Food was placed in the cage immediately after the operation.

The rats were paired according to body weight so that the average weight of the 'control group' would be approximately the same as that of the 'liver-fed group.' The 'control' and 'treated' groups were treated alike as regards operative technique.

Since it has been reported that starvation retards the regeneration of the rat's liver (6, 4, 7), and since Higgins and Anderson (5) observed that a control laparotomy would cause the loss of about 12 to 15 per cent of the body weight during 10 days, we believed it was necessary to control this factor. With this idea in mind the data from no rat have been used in making comparisons between the 'control' and 'liver-fed' groups if at 10.7 days more than 15 grams of weight had been lost.

TABLE 2. DATA ON PARTIALLY-HEPATECTOMIZED RATS SHOWING THE EFFECT OF FEEDING PIGS' LIVER FOR TEN DAYS

PROCEDURE	NO. OF RATS	AVR. BODY WEIGHT		AVR. WT. DRY LIVER AT NECR., 10.7 DAYS POSTOP.	AVR. WT. DRY LIVER REMOVED AT OPERATION	CALCUL. AVR. DRY WT. LEFT IN AT OP.	LIVER DRY WT. INCREMENT, FROM OP. TO NECR.
		At op.	At necr.		(× 0.46 =)		
Treated rats (fed liver)	22	217.9 ±7.2[1]	213.6 ±7.0[1]	2.276	1.356	0.624	1.652 ±0.06[1]
Control rats	29	215.3 ±5.6[1]	208.2 ±5.4[1]	1.782	1.386	0.638	1.144 ±0.03[1]
						Diff. =	0.508 ±0.09[1]

[1] Probable error of the mean.

$$\text{Formulae: P.E. mean} = 0.6745\,\frac{\sqrt{\frac{\Sigma(\delta)^2}{n}}}{\sqrt{n-1}}$$

where $\sqrt{n-1}$ is applicable for n >20, or < 30.

$$\text{P.E. diff.} = \sqrt{(\text{P.E.}_{\cdot m_1})^2 \pm (\text{P.E.}_{\cdot m_2})^2} = \pm 0.0716$$

$$\text{Critical Ratio (C.R.)} = \frac{\text{diff.}}{\text{P.E. diff.}} = 7.1$$

TABLE 3

PROCEDURE	NO. OF RATS	AVR. BODY WEIGHT		AVR. DRY WT. INCREMENT OF LIVER RESTORED	SIGMA	P.E.
		At op.	At necr.			
Controls	16	202.8	200.5	1.367 gm.	0.312	0.056
Wilson's conc. liver extr.	13	218.0	215.0	1.335[1]	0.434	0.088
Thiamine HCl	10	206.8	210.2	1.374[1]	0.309	0.077

[1] If these increments are compared to the control of 1.144 obtained on 29 rats in table 2, the difference is not significant.

The average results are shown in table 2. It is to be noted that the average body weight of the 29 control and 22 liver-fed rats was approximately the same at the time of the operation and at necropsy. However, the liver-fed rats regenerated 0.5 gram more dry liver substance than the control rats. This difference is statistically significant (C.R. = 7.1). The same is true of wet liver substance.

The effects of thiamine and a liver extract on the rate of regeneration. The significance of the foregoing observation is enhanced by the lack of effect of thiamine and of a liver extract used in the treatment of pernicious anemia (table 3).

In this experiment the rats were treated in exactly the same way as the foregoing group, except that all the rats received the stock diet, and that out of the 39 rats used, 16 served as a group of controls, 10 were given 200 micrograms of thiamine hydrochloride daily subcutaneously, and 13 were given subcutaneously 5 U.S.P. units of a concentrated liver extract which is used for the treatment of pernicious anemia (Wilson Laboratories, Chicago).

The ratio of dry-liver weight to wet-liver weight in the rats receiving the stock diet and those receiving pigs' liver. The significance of the favorable effect of pigs' liver is also supported by the data on the greater amount of dry substance accumulated by the regenerating liver cells of the rats fed pigs' liver (see table 4).

TABLE 4. RATIOS OF DRY LIVER WEIGHT TO WET LIVER WEIGHT

	RATS ON CONTROL DIET		RATS FED PIGS' LIVER	
	At operation	At necropsy	At operation	At necropsy
Wet wt.	$\frac{129.6}{26} = 4.985$	$\frac{181.3}{28} = 6.474$	$\frac{94.18}{20} = 4.709$	$\frac{148.49}{20} = 7.425$
Dry wt.	$\frac{40.303}{29} = 1.386$	$\frac{51.65}{29} = 1.782$	$\frac{29.83}{22} = 1.356$	$\frac{50.068}{22} = 2.276$
Ratios dry/wet	$\frac{1.386}{4.985} = 0.279$	$\frac{1.782}{6.474} = 0.275$	$\frac{1.356}{4.709} = 0.282$	$\frac{2.376}{7.425} = 0.306$

DISCUSSION

It is clear from the results of this experiment that a diet consisting solely of liver increases the rate of regeneration of the liver of a partially hepatectomized rat as compared to an ordinary stock diet which is adequate for the general nutrition of the body. The results also show that the active principle in the liver is neither thiamine nor the antipernicious anemia factor both of which are found in pigs' liver.

More definitive experiments will have to be performed to ascertain whether the effect of a liver diet on regeneration is due to a high protein intake, hepatic protein having a favorable amino acid composition or a growth-promoting substance other than thiamine and the antipernicious anemia factor.

CONCLUSION

Liver tissue, either because it contains a high quality protein or some as yet undetermined growth-promoting factor other than thiamine or the antipernicious anemia factor, facilitates the rate of regeneration of the liver in the partially hepatectomized rat.

REFERENCES

1. Mitchell, H. H. and T. S. Hamilton. Biochemistry of Amino Acids. Chem. Catalog Co., Inc., 419 4th Ave., N. Y.
2. McJunkin, F. A. and H. C. Breuhaus. Arch. Path. **12**: 901, 1931.
3. Marshak, Alfred and A. C. Walker. This Journal **143**: 226, 1945.
4. Brues, A. M., D. R. Drury, and Mildred C. Brues. Arch. Path. **22**: 658, 1936.
5. Higgins, G. M. and R. M. Anderson. Arch. Path. **12**: 186, 1931.
6. Higgins, G. M. and D. J. Ingle. Anat. Rec. **73**: 95, 1939.
7. Marchella, T. E., G. M. Higgins, and F. C. Mann. Am. J. Digest. Dis. **7**: 152, 1940.

METABOLIC RECOVERY RATES FROM EXERCISE AFTER ALTERATION OF ALKALINE RESERVE[1]

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Previous experiments have indicated that the limiting factors in respiratory gas exchange during recovery from exercise were probably circulatory in nature (1). This suggested the possibility that the CO_2 carrying capacity of the blood (or exchange capacities in the lungs and tissues) may be involved in the rate of CO_2 elimination. Accordingly some of the relationships between alkaline reserve and recovery rates from exercise have been studied.

A standard step-up exercise was used consisting of 20 steps per minute onto a nine-inch platform for three minutes. The methods for measuring CO_2 elimination and O_2 consumption after exercise were the same as those described previously(1). In brief the method consisted of continual and simultaneous measurements of CO_2 and O_2 in the expired air with thermal conductivity analyzers. Knowing the percentages of the gases and corresponding minute ventilations, the recovery curves for O_2 consumption and CO_2 elimination could be calculated.

The specific measurements obtained that describe the recovery period are: 1) recovery half-time constants of gas exchange, 2) metabolic gas exchange at zero recovery time and 3) O_2 debt and excess CO_2 eliminated during recovery. Half-time constants of recovery were obtained by plotting gas exchange recovery curves (above resting) semilogarithmically against time. These half-time constants represent rates of recovery; thus, a small or large time constant corresponds to a fast or slow rate of recovery, respectively. The metabolic gas exchange at zero recovery time was obtained by extrapolation of the exponential recovery curve to zero recovery time. This corresponds to A_0 in the general exponential recovery equation $A = A_0e^{-kt}$ where A is the gas exchanged at any time t, and k is the rate constant. O_2 debt and excess CO_2 were obtained by integration of the exponential recovery equation, which yields the general form: cc. of gas $= A_0KT$, where T is the half-time recovery constant in seconds and K is a conversion constant.

Control exercise tests were performed the day preceding the experimental tests. The latter tests were made 3 to 6 hours after ingestion of $NaHCO_3$ and 12 to 16 hours after ingestion of NH_4Cl. The majority of these tests were made on one experienced subject. No direct measurements of the alkaline reserve were made. However, it is assumed that ingestion of 20 grams of $NaHCO_3$ increases the

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alkaline reserve markedly (2). Likewise, NH₄Cl has been shown to decrease the alkaline reserve (2, 3).

Typical gas exchange recovery curves after ingestion of $NaHCO_3$, compared with control curves, are shown in figure 1. Since there are intra-individual variations in recovery rates, in addition to experimental errors, it was necessary to carry out a number of tests and make a statistical analysis of the data. Summarized in table 1 are results obtained on recovery CO_2 elimination following the increase in alkaline reserve by the ingestion of 20 grams of $NaHCO_3$ on five different days. A few hours after ingestion of the $NaHCO_3$ there was an average 23 per cent decrease in the time constants, which represents a 23 per cent increase in rate of recovery from exercise. Furthermore, the CO_2 elimination at zero recovery time, A_0, was increased by 26 per cent. On the other hand there was no significant change in the total excess CO_2 eliminated during the recovery period.

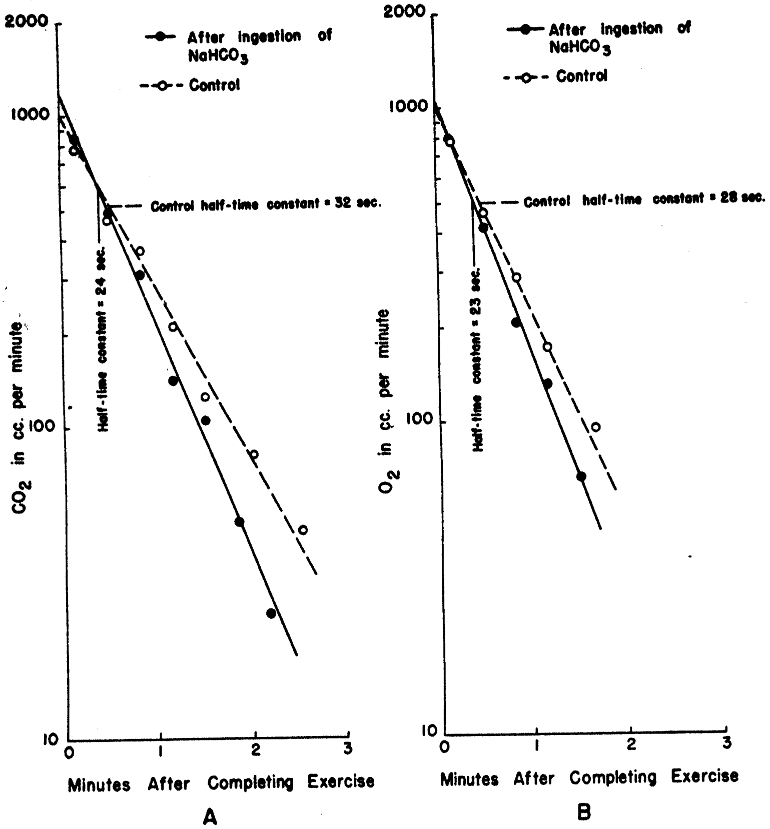

Fig. 1. SEMI-LOGARITHMIC plots of CO_2 elimination and O_2 consumption (above resting values) after ingestion of $NaHCO_3$ compared to control recovery curves. (Subject, W. E. B.) *A*. Effect of ingestion of $NaHCO_3$ on rate of recovery from moderate exercise as measured by CO_2 elimination. *B*. Effect of ingestion of $NaHCO_3$ on rate of recovery as measured by O_2 consumption.

In table 2 are summarized the data concerned with O_2 consumption during the recovery period. There was a significant 13 per cent decrease in the O_2 time constants, i.e., a 13 per cent increase in rate of recovery and a 9 per cent increase in O_2 consumption at zero recovery time; however, there was no significant change in the oxygen debt.

TABLE 1. EFFECT OF INGESTION OF $NaHCO_3$ ON CO_2 ELIMINATION DURING RECOVERY FROM EXERCISE

(Subject: W. E. B.)

METABOLIC MEASUREMENTS	CONTROL EXERCISE TESTS		EXERCISE TESTS AFTER INGESTION OF $NaHCO_3$		DIFFERENCE
	No. of tests	Average	No. of tests	Average	
Recovery half-time constants of CO_2 elimination (seconds)	39	31.1 ± 2.4	18	23.9 ± 2.8	23% decrease (t = 6.4, sig. at 1% level)
CO_2 eliminated at zero recovery time (cc./min. above resting)	39	866 ± 70	18	1090 ± 125	26% increase (t = 4.9, sig. at 1% level)
cc. excess CO_2 eliminated during recovery (above resting)	33	640 ± 45	14	618 ± 64	6% decrease (not significant)

TABLE 2. EFFECT OF INGESTION OF $NaHCO_3$ ON O_2 CONSUMPTION DURING RECOVERY FROM EXERCISE

(Subject: W. E. B.)

METABOLIC MEASUREMENTS	CONTROL EXERCISE TESTS		EXERCISE TESTS AFTER INGESTION OF $NaHCO_3$		DIFFERENCE
	No. of tests	Average	No. of tests	Average	
Recovery half-time constants of O_2 consumption (seconds)	34	27.3 ± 2.4	16	23.9 ± 1.1	13% decrease (t = 4.5, significant)
O_2 consumed at zero recovery time (cc./min. above resting)	34	970 ± 60	16	1060 ± 54	9% increase (t = 3.4, significant)
Oxygen debt (cc. O_2)	39	630 ± 41	18	595 ± 38	3% decrease (not significant)

Due to the unpleasant nature of the experiments these tests were made of one experienced subject. In an attempt to broaden the conclusions four additional subjects were used; two of these showed a 10 per cent decrease in their CO_2 recovery time constants after ingestion of 10–15 grams of $NaHCO_3$, and two showed no change. These results, however, are only tentative since one test was carried out per person and a statistical analysis is not possible.

In another series of tests on the experienced subject, the alkaline reserve was decreased by ingestion of 13 grams of enteric coated NH_4Cl tablets 12 to 16 hours before the exercise tests. This apparently had no effect on CO_2 recovery rates as indicated in table 3. There may have been an effect on the O_2 consumption recovery as there was a 7 per cent decrease in the time constants of O_2 consumption after ingestion of NH_4Cl.

TABLE 3. EFFECT OF NH_4Cl INGESTION ON RECOVERY RATES FROM EXERCISE (Subject: W. E. B.)

RECOVERY MEASUREMENTS	CONTROL EXERCISE TESTS		EXERCISE TESTS AFTER INGESTION OF NH_4Cl		DIFFERENCE
	No. of tests	Average	No. of tests	Average	
Half-time constant of CO_2 elimination (seconds)	39	31.1 ± 2.4	10	31.0 ± 2.2	1% decrease (not significant)
Half-time constant of O_2 consumption (seconds)	34	27.3 ± 2.4	10	24.2 ± 1.9	7% decrease (significant at 5% level)

DISCUSSION

Hill (4) and later Margaria *et al.* (5) distinguish two phases of recovery from an exhausting exercise. The oxygen consumption recovery curve after such an exercise is composed of an initial rapid exponential drop followed by a considerably slower exponential curve. The initial rapid phase is, according to Margaria, payment of an 'alactacid' oxygen debt and presumably represents energy for the resynthesis of phosphagen split during anaerobic muscular contraction. The slower recovery phase, or payment of the 'lactacid' oxygen debt, is concerned with oxidation of the accumulated lactic acid. Recovery from a moderate exercise is predominately or entirely repayment of the alactacid type of oxygen debt since there is no accumulation of lactic acid. In the present experiments it was decided to avoid complicating the recovery curves with two exponential phases, so the exercise chosen was of such mild intensity as to be followed only by the rapid recovery phase.

Previous evidence had indicated that the limiting factors operative during the rapid recovery phase were circulatory in nature and it was thought that alteration of alkaline reserve might conceivably influence this type of recovery, particularly as measured by the rate of elimination of carbon dioxide. As far as is known the effects of acidosis or alkalosis on the rapid recovery phase have not been studied. Dennig (6) and Margaria (5) concluded that alkalosis could improve the performance of exhaustive work by allowing greater accumulation of lactic acid, although Margaria found no effect on the velocity constants of the slower exponential recovery curve. The effect of alkalosis on the velocity constants of the rapid recovery phase was not determined.

These experiments, on one experienced subject, demonstrate that increasing the alkaline reserve by ingestion of $NaHCO_3$ significantly increases the rates of recovery from a mild exercise. The results lend additional support to the view that rates of metabolic gas exchange after a mild exercise are limited by circul tory factors. It seems probable that increased alkaline reserve results in a greater CO_2 carrying capacity of the blood which allows more rapid elimination of metabolic CO_2 from the muscles. Also, the increase in O_2 recovery rates may be related to altered CO_2 tensions which favor greater O_2 exchange capacities of the blood. Tests were carried out on a few other subjects, however at present there are insufficient data to determine the universality of these results.

The effect of alkalosis on recovery from an exhausting exercise was not tested, although, on the basis of the present results and on Margaria's results, one might expect that alkalosis would affect the velocity constants of the rapid recovery phase and would have no effect on the slower recovery curve.

Attempts to decrease recovery rates by lowering alkaline reserve were not successful; however, assuming a decreased alkaline reserve, reflected by a lowered CO_2 carrying capacity of the blood, there might have occurred a compensatory increase in the rate of blood flow, thus accounting for the slight increase in O_2 consumption recovery rates. On the other hand, there may be some question as to whether the amount of NH_4Cl used was sufficiently effective in lowering alkaline reserve.

These results suggest that intra- and inter-individual differences in recovery rates may be related in part to alkaline reserve.

SUMMARY

Tests carried out on one experienced subject showed that an increase of alkaline reserve by ingestion of 20 grams of $NaHCO_3$ brought about a 23 per cent increase of the rate of recovery from moderate exercise as measured by CO_2 elimination. There was a corresponding increase in the recovery rate as measured by O_2 consumption although to a lesser degree (13 per cent increase). Ingestion of NH_4Cl, with a presumable decrease in alkaline reserve, had no effect on the CO_2 recovery rate although a slight increase in the O_2 rate occurred. These results are interpreted as supporting the concept that the blood and circulation are limiting factors in metabolic gas exchange during recovery from a mild exercise.

The author is indebted to Miss Muriel Johnston for assistance in carrying out the experiments.

REFERENCES

1. Berg, W. E. This Journal **149:** 597, 1947.
2. Haldane, J. B. S. Lancet **1:** 537, 1924.
3. Baird, M. M., C. G. Douglas, J. B. S. Haldane, and J. G. Priestley. J. Physiol. **57:** xli, 1923.
4. Hill, A. V., C. N. H. Long and H. Lupton. Proc. Roy. Soc. B **84:** xcvii, 1924.
5. Margaria, R., H. T. Edwards, D. B. Dill. This Journal **106:** 689, 1933.
6. Dennig, H., J. H. Talbott, H. T. Edwards and D. B. Dill. J. of Clin. Invest. **9:** 601, 1931.

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EFFECTIVE OSMOTIC PRESSURE OF THE PLASMA PROTEINS AND OTHER QUANTITIES ASSOCIATED WITH THE CAPILLARY CIRCULATION IN THE HINDLIMBS OF CATS AND DOGS

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The principal factors regulating the exchange of fluid between capillaries and tissue spaces were outlined by Starling (1) following his experiments on the absorption of fluid from the connective tissue spaces. Starling's experiments, performed on perfused hindlimbs of dogs, gave rise to one of the most widely recognized hypotheses in physiology and medicine. According to the 'Starling Hypothesis', the direction and rate of fluid transfer between plasma and tissue fluids are determined by three factors; *a*) the hydrostatic pressures on each side of the capillary membranes, *b*) the protein osmotic pressures of plasma and tissue fluids acting across the capillary membranes, and *c*) the physical properties of the capillary membranes considered as mechanical filters.

Convincing quantitative evidence in support of the Starling theory was obtained by Landis (2) from direct measurements of the hydrostatic pressure and rates of fluid movement in individual capillaries of the frog's mesentery. In this preparation the rate of fluid movement across the capillary membrane is, on the average, proportional to the difference between the mean hydrostatic pressure in the capillary and the protein osmotic pressure of the plasma as measured in vitro. This behavior represents a special case of the Starling theory in which tissue pressure opposing filtration is negligible and the capillary membranes are almost completely impermeable to the plasma proteins; it implies that the protein concentration in tissue fluid immediately outside the capillary wall is too low to play a significant role in determining the osmotic balance.

No such direct measurements have been made in mammalian capillaries. Circumstantial evidence, reviewed by Landis (3), has in general lent support to the

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Starling theory, but the quantitative relations between capillary pressure, effective protein osmotic pressure and rate of fluid exchange have never been clearly demonstrated in the mammalian circulation. In the present paper we shall describe methods for measuring these variables in the isolated perfused hindlimbs of cats and dogs. Quantitative evidence will be given that the Starling theory is in fact applicable to the perfused hindlimb and with a precision rarely encountered in biological preparations.

METHODS

A. General. The hindlimbs of cats or dogs were amputated and suspended from a sensitive recording balance. They were supplied with blood from a pump-lung circulation under conditions such that the arterial perfusion pressure, the venous pressure and the protein osmotic pressure of the plasma could be independently adjusted to desired constant values. The rate of filtration of fluid from blood to tissues was recorded as the rate of gain of weight, a technique which has been employed in several previous investigations (4–6); conversely, absorption of fluid from tissues to blood was recorded as a loss of weight. The blood flow was measured and recorded continuously. From these quantities it is possible, by the methods described below, to determine the mean hydrostatic pressure in the capillaries, the effective osmotic pressure of the plasma proteins, the filtration coefficient of the capillary membranes and certain other quantities important to the fluid exchange.

B. Detail. 1. *Perfusion.* The technique of perfusion was similar to that described by Whittaker and Winton (7). A diagram of the perfusion circuit is shown in figure 1.

2. *Preparation of blood for perfusion.* Three hundred to 500 cc. of perfusion fluid were used for each experiment. The blood was drawn from the carotid arteries of one or more animals not more than three days prior to the experiment. For experiments with cats the blood was heparinized; with dogs the blood was usually defibrinated. When a high protein osmotic pressure was required, the plasma was separated from the cells and placed in cellophane bags in front of a fan. Loss of water by evaporation from the surface of the cellophane occurred at a rate sufficient to double the concentration of protein in about four hours. The bags were then sealed close to the liquid level and the concentrated plasma dialyzed against cold Ringer's solution (0.90% NaCl, 0.042% KCl, 0.024% $CaCl_2$, 0.020% $NaHCO_3$) until ionic equilibrium was established as indicated by the electrical conductivity. The pooled plasma concentrates from several cats were required for experiments involving high protein pressures. Low protein pressures were obtained by diluting whole blood with Ringer's solution. It was noted that the protein osmotic pressure of plasma obtained by 'bleeding out' from the carotid arteries was considerably less than normal, the average pressure being 15 mm. Hg. Presumably this was the result of dilution of the plasma with tissue fluid during the bleeding process.

The corpuscular concentration was adjusted to desired levels by the addition of red cells to the previously prepared plasma. The whole fluid was filtered through glass wool and two layers of linen cloth before admission to the perfusion reservoir.

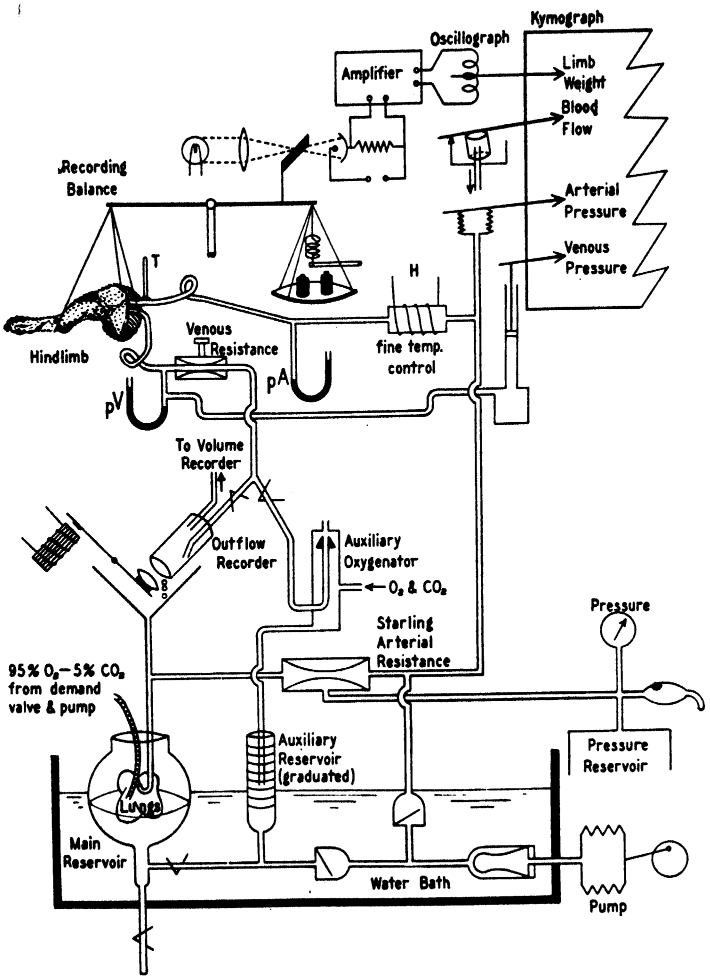


Fig. 1. Diagram of the perfusion circuit and weight-recording system. The pump-lung-hindlimb circulation is similar to that employed by Whittaker and Winton (7). The leg is mounted horizontally on the balance with the ventral surface facing upwards. The balance beam axle rotates on ball bearings. The beam is loaded with a spring which lengthens 0.21 mm. per gram; optical and electrical amplification of this displacement allow full scale deflection (50 mm.) of the recording oscillograph for a change of one gram on the balance. Temperature of blood in the arterial cannula is measured by thermometer (T) and controlled by heating coil (H).

3. *Operative procedures.* All experimental animals were anesthetized with nembutal (40 mgm. per kgm.). The right hindleg from a small dog (5 to 6 kgm.) or a large cat (2.8 to 4 kgm.) was separated from the body at the hip, all visible blood vessels being ligatured except the femoral artery and vein. Small bleeding

points were seared with a cautery. The operation required one and one-half to two hours. The femoral vein was cleared of connective tissue as far down as the saphenous branch; this was necessary to prevent compression of the vein from the weight of unsupported muscles pulling on the tissue immediately adjacent to the vein.

During this operation the lungs were removed from a second animal and the pump-lung circulation started about one-half hour before the preparation of the leg for perfusion was complete. Arterial and venous perfusion cannulae of sizes appropriate to the individual vessels were fitted to the perfusion apparatus and calibrated as described in section 4 below. The femoral artery and vein were then cut and the leg transferred to the perfusion apparatus: transfer from the natural to the artificial circulation required about two minutes.

The circulation through the leg prepared in this way was such that in the first hours of perfusion all the perfusing blood left the leg via the femoral vein. There was no detectable drainage from the lymphatics. Towards the end of each experiment, however, a small amount of blood (less than two per cent of total flow) left the leg by oozing from small vessels which had been cut during the operation. The protein content and corpuscular concentration of this blood were indistinguishable from those of venous blood in the two instances in which these quantities were compared. When dye was injected into the arterial perfusion cannula the whole leg rapidly became colored up to the cut surfaces of the skin and muscle.

4. *Pressures.* The mean arterial and venous pressures were determined with mercury and blood manometers, respectively. The manometers were connected to the perfusion circuit at some distance from the arterial and venous perfusion cannulae so that it was necessary to correct for the pressure drop across the tubing and cannulae in order to obtain the true pressure in the artery and vein at each flow rate. Since a change of 0.5 mm. Hg in mean capillary pressure produced an easily detectable change in rate of fluid movement, it was necessary to devote considerable attention to these corrections. The pressure-flow characteristics of each cannula were obtained before and after each experiment by substituting a short length of wide bore tubing for the leg and determining the pressure difference across the cannulae at various flow rates in the range used during the experiment. Pressure-flow diagrams were then constructed for each cannula and the appropriate corrections for each flow rate during the experiment derived from the smoothed curves. The true zero of each manometer was determined by extrapolating the indicated pressure-flow diagram to zero flow. All pressures are referred to the level of a horizontal plane bisecting the leg. The uncertainty in determining this level, and hence the absolute value of all hydrostatic pressures, was about ±.5 mm. Hg.

5. *Blood flow.* The rate of venous outflow was measured at intervals by a stopwatch and measuring cylinder; it was also recorded simultaneously by a Gaddum (8) outflow recorder.

6. *Weight.* The center of the balance beam was fitted with a steel axle which rotated on ball bearings. One arm of the balance was loaded with a helical spring

which lengthened 0.21 mm. per gram. The main weight of the limb was balanced with calibrating weights, as indicated in figure 1, and *changes* in weight were recorded from the small off-balance excursions of the spring-loaded arm. The excursions were amplified approximately 100-fold and were recorded on the kymograph as follows: one arm of the balance was provided with a shutter which interrupted light focussed on a photo tube (Cetron CE-2). The output of the photo tube was led through an impedance changer (6SJ7) and transconductance stage (6AG7) to a Weston Model 30 moving coil relay modified as described by Winton (9) to operate as a recording oscillograph. Stabilizing devices were employed for all voltage supplies so that at equilibrium temperatures the overall drift in the recording system corresponded to a change of less than two mgm. per minute on the balance. The sensitivity of the system was controlled electrically and was generally operated so that one gram on the balance produced a deflection of 20 to 30 mm. on the kymograph. The deflection of the recorder was linearly related to the weight and was calibrated at intervals during the experiment by adding gram weights to the balance. The rubber tubing which connected the perfusion cannulae on the balance to the perfusion system did not interfere with the measurements, owing to the small displacement of the balance (0.4 mm.) required for full scale deflection of the recorder. The leg itself lay horizontally with the flat dorsal surface supported by a malleable coarse mesh wire screen which was attached to the balance arm by means of three chains of adjustable length.

7. *Temperature control.* Blood in the arterial cannula was maintained at 37.5°C. at all flows by means of a heater coil (H, fig. 1) operated from a manually controlled variable transformer.

8. *pH.* The pH of plasma or of protein solutions was measured with a glass electrode (Beckman).

9. *Protein osmotic pressure.* In vitro measurements of protein osmotic pressure were made with a Hepp osmometer (10) as modified by Brown (11). In our hands multiple determinations of osmotic pressure performed on samples of the same protein concentration agreed within a standard deviation of ±0.7 mm. Hg. The absolute values of protein osmotic pressure found at any given protein concentration and pH agreed with the values calculated from the data of Scatchard, Batchelder and Brown (12, 13) as indicated in table 1. Calculation of the molecular weight of albumin from the observed osmotic pressures of bovine albumin lead to a value of 69,000, which is in close agreement with values obtained by independent methods (14). The measurements were made against 0.15 molar NaCl at room temperature with the plasma or protein samples in equilibrium with ambient air. Since the samples in vivo were equilibrated with a pCO_2 of 40–45 mm. Hg, the pH was higher in the osmometer than in the capillary circulation. The effects of a change in pH from 7.2 to 7.7 may be predicted from the equation of table 1; they were too small to be detected with our apparatus and are not considered in the final figures. However, all data were corrected to 37°C., the observed values being multiplied by the factor 310 ÷ (273 + t°C.). The actual temperature in the capillaries during the perfusion was less than 37°C. by

an amount which varied with the rate of blood flow; an error not exceeding +2 per cent may arise from neglect of this factor.

EXPERIMENTAL

1. *The effects of arterial pressure on the limb weight.* Figure 2 shows the effects of variations in arterial pressure on the limb weight and blood flow, the venous pressure and the protein pressure remaining constant. The arterial pressure was initially set to maintain the limb at constant weight. A sudden rise from 91 to 110 mm. Hg produced an initial increment of weight followed by a slow steady gain in weight. Presumably the initial rise is a result of an increased vascular volume beyond the arterial system, for it is abolished if the arterioles are constricted with adrenaline. Nor is it present if the capillary pressure is maintained constant by simultaneous lowering of the venous pressure as shown in figure 4. The slow steady gain in weight (+0.26 grams/min.) is presumably a result of filtration of fluid from blood to tissues. When the arterial pressure was restored to its initial value, the vascular volume was also restored, following which the leg weight remained constant at a new level corresponding to the amount of fluid which had been filtered. The arterial pressure was then lowered below the value necessary to maintain constant weight. Exactly analogous changes of weight resulted except that they were of opposite sign. The slow steady loss of weight

TABLE 1. COMPARISON OF PROTEIN CONCENTRATION WITH PROTEIN OSMOTIC PRESSURE

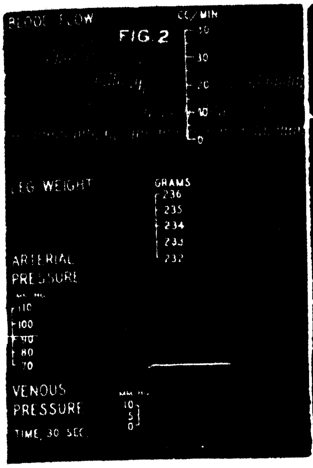
SAMPLE	[1]PROTEIN CONCENTRATION	PROTEIN OSMOTIC PRESSURE, MM. Hg	
		Observed	[2]Calculated from protein concentration
	grams/100 cc.		
1. Cat plasma	4.8	13.6 ±.7	14.0
2. Bovine albumin pH 5.7	4.1	14.7 ±.2	14.3
3. Cat plasma	5.2	16.2 ±.6	16.0
4. Cat plasma	6.0	20.8 ±.8	20.6
5. Cat plasma + bovine albumin	8.7	45.3 ±.8	50.1

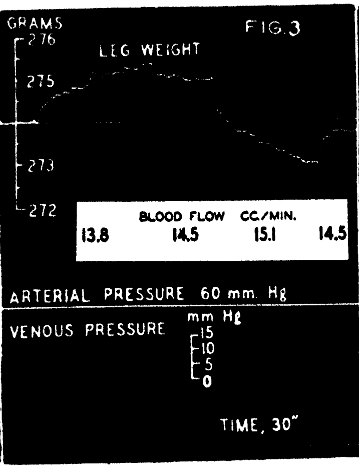
[1] Calculated from protein nitrogen with exception of sample 2 which was estimated from the density. We are indebted to Dr. Hegsted and members of the Department of Nutrition, Harvard Medical School, for performing the Kjeldahl analyses.

[2] Calculated from the equation of Brown, Batchelder and Scatchard (12) as modified by Scatchard (15):

$$\pi = \frac{268(1 - 0.64g)c}{1 - (0.4 + 0.9\text{pH})c}$$

where π = protein osmotic pressure, mm. Hg; c = protein concentration, g./cc; g = ratio of globulin to total protein. We assumed that in normal cat plasma $g = 0.42$, corresponding to an $A:G$ ratio of 1.4. Electrophoretic studies of Deutsch and Goodloe (16) indicate that the actual $A:G$ ratio in cat plasma is 0.7. However, a large proportion of the globulin fractions are proteins of low molecular weight which produce the osmotic equivalent of a higher $A:G$ ratio. Kjeldahl analyses of the above samples after precipitation with Na_2SO_4 yielded 'apparent $A:G$ ratios' of 1.3–2.0.





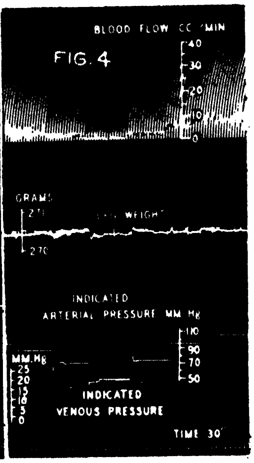


FIG. 2. THE EFFECTS OF ARTERIAL PRESSURE ON LIMB WEIGHT. A rise of arterial pressure above the isogravimetric value (91 mm. Hg) produced a sudden increase in limb weight (increased vascular volume) followed by a slow rate of gain of weight of +0.26 gram per minute (filtration rate). Exactly analogous changes, but of opposite sign, followed a fall in arterial pressure below the isogravimetric value (absorption rate −0.28 gram per minute). The time required to complete changes of vascular volume is variable and must be considered in determining the final slope. Slight alterations in venous pressure occurred owing to the changed pressure drop across the venous cannula at each different rate of blood flow; corrections for this variation are made as described in text. The arterial pressure scale is corrected for the pressure drop across the arterial cannula.

FIG. 3. THE EFFECTS OF VENOUS PRESSURE ON LIMB WEIGHT. A rise of venous pressure produced an initial small increment in limb weight (vascular volume) followed by a slow sustained rate of gain of weight of +0.17 gram per minute (filtration rate). Exactly analogous changes, but of opposite sign, followed a fall in venous pressure (absorption rate −0.21 gram per minute). The arterial and protein osmotic pressures remained constant. The time required to complete changes of vascular volume is variable and must be considered in determining the final slope. Note that the alterations in venous pressure, although small, produced detectable and reversible changes of blood flow as well as easily measurable rates of fluid transfer.

FIG. 4. THE ISOGRAVIMETRIC STATE. This record illustrates the method of obtaining isogravimetric values for arterial pressure and blood flow. The tendency for each stepwise decrement in arterial pressure to cause absorption (as in fig. 2) is compensated for by increasing the venous pressure until the limb weight remains constant. In order to maintain the weight as constant as illustrated it was necessary to adjust the venous pressure within 0.3 mm. Hg and the arterial pressure within 2 mm. Hg. The isogravimetric capillary pressure and postcapillary resistance to blood flow are determined from the isogravimetric blood flow and the isogravimetric venous pressure as illustrated in figure 5. Indicated pressures must be corrected for the pressure drop across the cannulae at each rate of blood flow. Note that there are no changes of vascular volume such as those found with an uncompensated change of either arterial or venous pressures (figs. 2 and 3). This suggests that the principal site of change in vascular volume is the capillary bed; alternatively, changes in volume on the arterial side of the capillary circulation must be exactly counterbalanced by the changes in volume on the venous side of the capillary circulation.

(−0.28 grams/min.) is presumably a measure of the rate of absorption of fluid from tissues to blood.

DISCUSSION

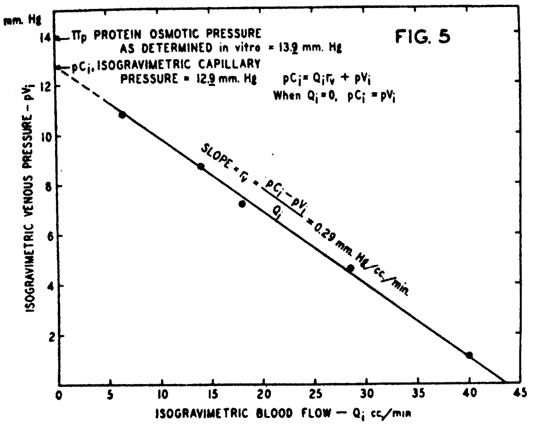
In the intact animal changes of arterial pressure are generally associated with complex changes in mean capillary pressure owing to simultaneous alterations in vascular tone. It is therefore not surprising that in the whole animal a rise of arterial pressure may cause filtration (17), absorption (18) or no detectable change in fluid balance (19). The results illustrated in figure 2 and in the remaining sections of this paper clearly demonstrate the quantitative relations between arterial pressure and rate of filtration or absorption when all other known factors are maintained constant.

2. *The effects of venous pressure on the limb weight.* Figure 3 shows the effects of variations in venous pressure on the limb weight, the arterial and protein pressures being maintained constant. The effects are similar to those produced by arterial pressure except that the changes of venous pressure required to produce comparable rates of filtration or absorption are one-fifth to one-tenth as great (compare fig. 3 with fig. 2). Thus an easily measurable rate of filtration is brought about by a rise of 4 mm. Hg in the venous pressure and a detectable effect results from a change of 0.5 mm. Hg or less.

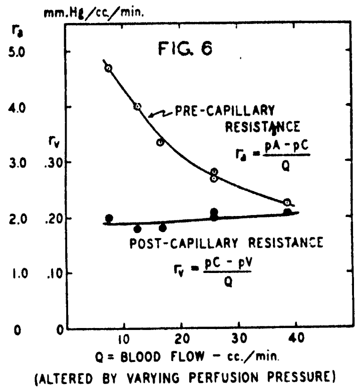
3. *The 'isogravimetric state.'* It will be clear from paragraphs 1 and 2 above that there exist an infinite number of pairs of values of arterial and venous pressures at which the leg will remain at constant weight. Thus the tendency to absorb, caused by a given reduction in arterial pressure, can be counterbalanced by raising the venous pressure until no net transfer of fluid occurs between blood and tissue (constant weight). Such an experiment, in which seven pairs of 'isogravimetric' arterial and venous pressures were obtained, is illustrated in figure 4. It is evident that if the arterial pressure is progressively lowered and the venous pressure raised to maintain constant weight there will come a point at which the venous pressure will equal the arterial pressure. Under these conditions there will be no pressure drop along the vascular tree and both the arterial and venous pressures will equal the capillary pressure. Since, by the terms of the experiment, there is no net transfer of fluid, this value of capillary pressure (isogravimetric capillary pressure) is equal and opposite to the sum of all pressures opposing filtration.

As pointed out by Green (20) the mean capillary pressure will be slightly less in the upper (ventral) half of the preparation than in the lower half. There will therefore be some transfer of fluid in the isogravimetric state even when the arterial and venous pressures are nominally equal. The average thickness of the hindlimb is about 2.5 cm. The mean capillary pressure in the top half is therefore about 0.5 mm. Hg less than in a horizontal plane bisecting the leg. This difference is by no means trivial since a change of 0.5 mm. Hg in mean capillary pressure regularly produces a detectable change in rate of fluid exchange in this preparation (section 2, fig. 9, table 3). In the isogravimetric state we therefore suppose that absorption occurs in the upper half of the preparation at a net rate of about 6 mgm. per minute per 100 grams tissue, while an equal rate of filtration takes place in the lower half even when arterial and venous pressures are nominally equal.

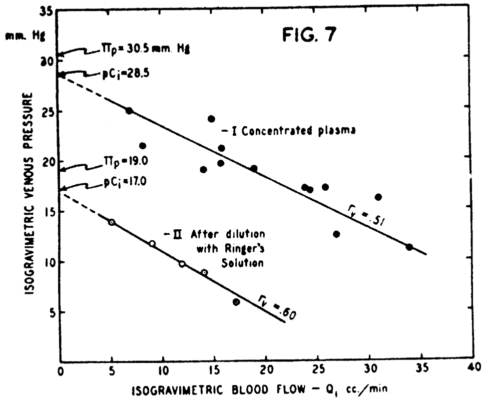
The isogravimetric capillary pressure cannot be obtained directly by equalizing arterial and venous pressures without disturbing the physiological properties of the capillaries because, when the arterial and venous pressures are equal, there is



DETERMINATION OF ISOGRAVIMETRIC CAPILLARY PRESSURE AND POST-CAPILLARY RESISTANCE TO BLOOD FLOW. Isolated hindlimb of cat, Exp. 23.



ANOMALOUS FLOW IN PRE-CAPILLARY CIRCULATION
Hindlimb of cat, Exp. 25
Hematocrit = 32%

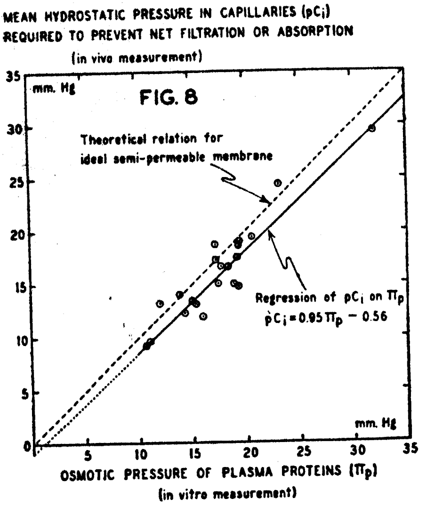


EFFECT OF PROTEIN CONCENTRATION ON ISOGRAVIMETRIC CAPILLARY PRESSURE – pC_i
(Hindlimb of cat, Exp C-15)

FIG. 5. THE DETERMINATION OF ISOGRAVIMETRIC QUANTITIES. Each point on the graph was obtained as illustrated in figure 4—the blood flow was altered by varying the arterial pressure and the venous pressure adjusted to maintain constant weight. At zero flow the pressure is everywhere equal along the vascular tree and, by the terms of the experiment, this pressure is equal and opposite to the sum of all pressures opposing filtration. At all finite values of blood flow the pressure at the arterial end of the capillary bed is greater than at the venous end; however, no *net* transfer of fluid occurs and the *mean* capillary pressure is presumably the same at all blood flows. The resistance to blood flow from the effective midpoint of the capillaries to the vein is, by definition, equal to the slope of the observed line; in contrast to precapillary resistance it is independent of rate of flow.

FIG. 6. THE RESISTANCE TO BLOOD FLOW from the femoral artery to the effective midpoint of the capillary circulation (precapillary resistance) increases as the flow is reduced by lowering the arterial pressure. This phenomenon may be a result of anomalous viscous flow of blood in the arterioles; the effect is diminished when the arterioles are dilated (21) or when most of the corpuscles are removed (7, 21) as described in text. The resistance to flow on the venous side of the capillary circulation (postcapillary resistance) is substantially independent of the flow rate.

no blood flow. However, its value may be estimated by plotting the difference between isogravimetric arterial and venous pressures (abscissa) against the isogravimetric arterial or venous pressures (ordinates) and extrapolating to zero pressure difference. The intercept on the ordinate is then the isogravimetric capillary pressure.



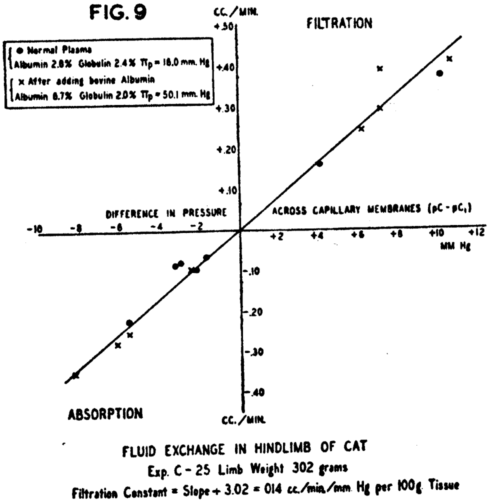


FIG. 8. THE EFFECTIVE OSMOTIC PRESSURE OF THE PLASMA PROTEINS in the hindlimb capillaries of cats and dogs. The mean hydrostatic pressure in the capillaries required to prevent net transfer of fluid is slightly less than the (in vitro) osmotic pressure of the plasma proteins over the entire range of protein concentrations so far investigated. The mean difference between these two quantities is 1.7 mm. Hg as compared with a standard error of measurement of 0.54 mm. Hg. In all probability this difference arises from a small concentration (0.7 ± .2%) of protein in tissue fluid bathing the external surfaces of the capillaries as explained in the text. At normal plasma protein pressures (23–27 mm. Hg) the effective osmotic pressure acting across the capillary membranes is 93 ± 1.5 per cent of the protein pressure as measured across a collodion membrane in vitro.

FIG. 9. FLUID EXCHANGE IN THE HINDLIMB OF A CAT. The rate of fluid exchange is simply proportional to the difference between the mean hydrostatic pressure in the capillaries ($pC = Qr_v + pV$) and the sum of all pressures opposing filtration (isogravimetric capillary pressure, pC_i); it is independent of the absolute value of these quantities. The results of figures 8 and 9, taken together, show that the Starling Hypothesis may be applied with some precision to the hindlimbs of cats and dogs perfused under the specialized conditions of these experiments.

In practice this method of determining the isogravimetric capillary pressure is not the most precise one owing to the fact that the extrapolation is alinear. This alinearity will be discussed in greater detail below; it arises from the complex relations between pressure and flow which obtain when whole blood is employed for perfusion. The difficulty may be avoided by considering the relations between isogravimetric blood flow and isogravimetric venous pressure in the following analysis.

4. *The determination of capillary quantities.* Definition of symbols: the following notation will be employed in the remaining sections of this paper.

pA = arterial pressure, mm. Hg
pV = venous pressure, mm. Hg
pC = mean capillary pressure, mm. Hg
π_p = osmotic pressure of plasma proteins, mm. Hg
π_t = osmotic pressure of proteins in tissue fluid, mm. Hg
Q = blood flow, cc./min.
r_a = $(pA - pC) \div Q$ = resistance to blood flow from artery to effective midpoint of capillaries (pre-capillary resistance).
r_v = $(pC - pV) \div Q$ = resistance to blood flow from effective midpoint of capillaries to vein (post-capillary resistance).
i = subscript referring to isogravimetric conditions.
$\pm F$ = rate of filtration (+) or absorption (−), grams/min.

Rearranging the definition of r_v we have, in the isogravimetric state

$$pC_i = Q_i r_v + pV_i$$

In order to solve for pC_i, plot Q_i (abscissa) against pV_i (ordinate) from data similar to that shown in figure 4. Extrapolate to zero flow; when $Q_i = 0$, $pC_i = pV_i$. Such a plot is shown in figure 5. It is seen that the relation between Q_i and pV_i is linear over a wide range of values and that the extrapolation to zero flow may be made with an uncertainty of less than 1 mm. Hg. The slope of the line is r_v, which is constant and independent of flow.

Presumably both filtration and absorption occur at all finite values of blood flow in the isogravimetric state. Thus when pV_i is small and Q_i large (fig. 5), the pressure drop along the capillaries is also large; filtration occurring at the arterial ends or along the entire length of some capillaries is counterbalanced by equal absorption at the venous ends or along the entire length of other capillaries. But as the flow is reduced the pressure drop in the capillaries is also reduced until, at zero flow, the pressure is everywhere equal along the capillary and no fluid exchange takes place along its length. It is of importance for the subsequent development in this paper to note that the value of r_v is unaltered by the changes of flow or of filtration and absorption.

A similar analysis can be made on the arterial side of the capillary circulation using the definition $pC_i = pA_i - Q_i r_a$. In this case, however, it is found experimentally that the relation between Q_i and pA_i is not linear. Since pC_i is constant (isogravimetric conditions) it follows that r_a, the precapillary resistance to blood flow, must vary with flow. This is illustrated in the experiment of figure 6 in which the precapillary resistance was more than doubled as the flow was decreased by reducing the perfusion pressure. The postcapillary resistance (r_v), determined simultaneously, remained constant.

DISCUSSION

This increase in precapillary resistance at low flows is not unexpected. It was found by Pappenheimer and Maes (21) and by Green *et al.* (22) that the overall resistance to blood flow ($r_a + r_v$) increases greatly at low flows and that the effect is most prominent in constricted vessels. The former workers attributed the effect to the anomalous viscous properties of the blood, for it was not present when Ringer's solution was substituted for blood. The data now presented (fig. 6) show that the entire effect is localized in blood vessels proximal to the effective midpoint of the capillaries. It may be inferred that the blood vessels on the venous side of the capillary circulation have dimensions such that anomalous flow effects are too small to be of significance; in this respect they resemble fully dilated arterioles. Six of the present series of experiments have been conducted with plasma containing relatively few corpuscles (hematocrit about ten per cent). Under these conditions the precapillary resistance (r_a) varied only slightly with changes of flow, thus confirming and extending the earlier observations with Ringer's solution.

While the resistance to blood flow is not the principal subject of this paper, it may be pointed out that the resistances to flow on either side of the capillary circulation have not previously been measured, although they are major factors determining the mean capillary pressure and the rate of fluid transfer as pointed out by Bayliss and Starling (23). The dependence of mean capillary pressure on arterial and venous tone may be stated quantitatively by combining the definitions of r_a and r_v given above and solving for pC.

$$pC = \frac{\frac{r_v}{r_a} pA + pV}{1 + \frac{r_v}{r_a}}$$

It is seen that at any given values of arterial and venous pressures the mean capillary pressure depends solely on the *ratio* of the postcapillary to precapillary resistances to blood flow. Since r_v and r_a are now measurable the way is open for the quantitative study of factors affecting the mean capillary pressure.

5. *Comparison of the isogravimetric capillary pressure with the osmotic pressure of the plasma proteins.* The isogravimetric capillary pressure determined as described above is equal to the sum of all pressures opposing filtration. It is of interest to compare its value with the osmotic pressure of the plasma proteins as determined with an artificial semipermeable membrane in vitro. Figure 7 shows the effects of alterations in protein concentration on isogravimetric quantities in one experiment. It is seen that the protein osmotic pressure as measured in vitro (π_p) was approximately 2 mm. higher than the isogravimetric capillary pressure at each of the two protein concentrations employed. Note that the postcapillary resistance to blood flow (r_v) remained approximately constant throughout the measurements; alterations in postcapillary resistance, produced by the infusion of drugs or other procedures, do not affect the value of pC_i if the protein concentration is constant.

The results of 22 such comparisons of pC_i with π_p are summarized in figure 8. The upper (broken) line represents the theoretical values which would obtain if the isogravimetric capillary pressure were equal and opposite to the (in vitro) protein pressure. The lower solid line is the best straight line drawn through the experimental points (method of least squares). It is seen that the capillary hydrostatic pressure in the isogravimetric state is, on the average, one to 2 mm.

Hg less than the protein pressure as measured in vitro over the range of protein pressures so far investigated. Thus,

$$pC_i = 0.95\pi_p - 0.56 \qquad S.D. = \pm .6 \text{ mm. Hg}$$
$$S.E. = S.D. \div \sqrt{n} = 0.34 \text{ mm. Hg.}$$

If no other forces (e.g., tissue pressure) were involved, the results of figure 8 could be explained on the basis of a concentration of protein in tissue fluid sufficient to exert an osmotic pressure of 1 mm. Hg in the lower range of protein concentrations and 2 mm. Hg in the higher range. It may be noted that tissue pressure would act to increase pC_i with respect to π_p. Thus if the protein concentration were reduced to zero we should expect that a *positive* hydrostatic pressure in the capillaries would be required to counterbalance tissue pressure. Actually the extrapolation to zero protein pressure (fig. 8) intercepts the capillary pressure axis at a negative value (−0.6 mm. Hg), a result which would be difficult to explain in terms of tissue pressure but which would be expected if a small concentration of protein remained in the tissue fluid. Further evidence will be given below (Section 6B) that the tissue pressure is in fact negligible under the conditions of these experiments and we make the provisional conclusion that the isogravimetric capillary pressure is equal and opposite to the effective osmotic pressure of the plasma proteins.

$pC_i = (\pi_p - \pi_t)$ = effective protein pressure across capillary membranes.

If this conclusion is correct we have for normal plasma ($\pi_p = 25$ mm. Hg) $\frac{pC_i}{\pi_p} = \left(0.95 - \frac{.56}{25}\right) = 0.93$, whence the effective osmotic pressure of the plasma proteins within the living capillaries is 93 per cent (standard error ±1.5 per cent) of the osmotic pressure measured with a collodion membrane in vitro. The mean value of π_t under these conditions is 1.7 ± 0.4 mm. Hg, a pressure which would be expected in fluid containing 0.8 ± .2 grams plasma protein per 100 cc. This estimate of protein in tissue fluid is too high if albumin and low molecular weight globulins constitute the principal protein fractions of the capillary filtrate. Electrophoretic studies of serum exudates (24) and of lymph (25) suggest that a partial sieving action does occur, and in this case our estimate of protein in fluid bathing the capillary walls would be closer to 0.7 ± 0.2 per cent.

The close correspondence between isogravimetric capillary pressure and plasma protein pressure is maintained only in the first few hours of perfusion and the data given above were obtained in this period. After 4 to 6 hours the isogravimetric capillary pressure diminishes; in a typical experiment it remained within 90 to 95 per cent of the plasma protein pressure for the first four hours of perfusion, but fell to 70 per cent in the fifth hour and to 30 per cent after six hours of perfusion. Presumably this is a quantitative expression of failure of the capillary membranes to retain plasma protein after prolonged perfusion.

DISCUSSION

The isogravimetric capillary pressure is, on the average, 1.7 ± 0.4 mm. Hg less than the osmotic pressure of the plasma proteins and this value is equivalent to a protein concentration of 0.7 ± .2 per cent in fluid bathing the external surfaces of the capillaries. Inspection of table 2 shows that this concentration is slightly greater than estimates of protein in capillary filtrate (26) or in lymph obtained during venous congestion (27). On the other hand it is considerably less than the protein concentration in small samples of lymph collected during massage of the resting dog's leg (28). It is clear that these results do not confirm the view, originally expressed by Starling (1) and repeatedly stated by Drinker (29–31), that the composition of tissue fluid is identical with that of lymph. Indeed, it appears unlikely that the protein concentration of tissue fluid is at any time uniform throughout the interstitial spaces. The diffusion coefficients of the plasma proteins are such that relatively large concentration gradients are possible between tissue fluid undergoing absorption at the venous end of the capillaries and tissue fluid composed of freshly formed capillary filtrate at the arterial end of the capillary. Even if all filtration and absorption processes were stopped, some 20 minutes would be required to reach 90 per cent equalization of protein concentration over a distance of 50 microns. As pointed out by Landis *et al.* (26), the protein content of lymph may lie anywhere between that of capillary filtrate (as in venous congestion) and that of tissue fluid immediately adjacent to capillary areas taking part in the absorptive process. On this hypothesis our estimate of protein in tissue fluid represents an average concentration lying between these two extremes.

TABLE 2. PROTEIN COMPOSITION OF TISSUE FLUIDS

FLUID	PROTEIN	NUMBER OF OBSERVATIONS	REFERENCES
1. Capillary filtrate in human forearm during venous congestion (60 mm. Hg)	$c = 0.3 \pm 0.3\%$	4	(26)
2. Lymph from dog's leg during venous congestion (40 to 60 mm. Hg)	$c = 0.2\%$	1	(27)
3. *Average* tissue fluid bathing external capillary surface in perfused limbs of cats and dogs	$\pi = 1.4 \pm 0.4$ mm. Hg. $c = 0.7 \pm 0.2\%$	22	Present data
4. Lymph obtained during massage of quiescent limb (dog)	$\pi =$ [1]$7.5 \pm .8$ mm. Hg. $c = 1.9 \pm 0.2\%$	8	(28)

[1] This value is larger than would be predicted from the observed protein concentration. The osmotic pressure of a 1.9 per cent solution of pure albumin in .15 molar NaCl at pH 7.3 is 6.1 mm. Hg (13) and that of a 1.9 per cent solution of plasma proteins is less than 5.0 mm. Hg.

6. *Filtration and absorption.* A. THE FILTRATION COEFFICIENT. Once having determined r_v in the isogravimetric state, it is possible to compute the value of the mean hydrostatic pressure in the capillaries when the arterial or venous pressures are altered so as to produce *net* filtration or absorption as in figures 2 and 3. Thus the value of r_v was found to be independent of the blood flow (figs.

5, 6 and 7) and of changes in filtration or absorption of fluid along the capillary wall (section 4). The mean hydrostatic pressure in the capillaries following an uncompensated change of arterial or venous pressure is then given by the relation

$$pC = Qr_v + pV$$

where r_v is obtained in the isogravimetric state but Q and pV are no longer isogravimetric quantities.

The mean pressure head across the capillary membranes available for filtration or absorption is $pC - pC_i$ for the latter term is equal to the sum of all pressures opposing filtration. Figure 9 shows the relation between the pressure head across the membranes and the rates of net filtration ($+F$) or net absorption ($-F$). The rate of fluid exchange is simply proportional to the pressure difference across the capillary membranes. Black circles are points obtained with undiluted blood with a measured osmotic pressure of 16.0 mm. Hg. The crossed points were obtained after concentrated bovine albumin was added until the protein osmotic pressure was 50.1 mm. Hg. The two sets of points do not differ significantly.

TABLE 3. FILTRATION COEFFICIENTS OF PERFUSED HINDLIMBS

SPECIES	FILTRATION COEFFICIENT K = GRAMS/MIN/MM Hg/100 GRAMS TISSUE		
	Mean	Error of mean	Standard deviation
Cats (14)	0.012	0.0007	0.0027
Dogs (4)	0.014	0.0006	0.001

The rate of fluid exchange is therefore independent of the absolute values of capillary and protein pressures and depends only on the *difference* between the mean capillary pressure (pC) and the effective protein osmotic pressure (pC_i). Thus,

$$\pm F = K(pC - pC_i)$$

where the proportionality factor (K) may be termed the 'filtration coefficient' of the capillary membranes. The mean value of K in the hindlimbs of 14 cats was 0.012 grams per minute per mm. Hg pressure difference across the capillary membranes per 100 grams tissue. The value was not significantly different in the hindlimbs of four dogs (table 3).

DISCUSSION

The results of figure 9 show that the rate of flow of fluid passing in either direction across the capillary membranes is simply proportional to the difference between the mean hydrostatic pressure in the capillaries and the sum of all pressures opposing filtration. Evidence has been given above that the latter term is equal to the effective osmotic pressure of the plasma proteins. These results, taken together, show that the Starling Hypothesis may be applied with some precision to the hindlimbs of cats and dogs perfused under the specialized conditions of these experiments.

The values of table 3 may be compared with estimates of filtration rate in the intact human forearm. Landis and Gibbon (32) found that a rise in venous pressure of 10 cm. H_2O produced, on the average, a filtration of 0.033 cc. per 100 cc. forearm per minute. Assuming that a rise in venous pressure of 10 cm. H_2O produces an increment of 8 cm. H_2O in mean capillary pressure, the 'filtration coefficient' of the human forearm would be 0.0055 gram/min/mm. Hg/100 grams tissue or less than half that of the isolated perfused hindlimb of the cat or dog. This difference may be an expression of a smaller capillary surface per unit volume of tissue in the human forearm, a difference which is suggested also by the absolute value of the blood flow, which is generally reported to be less per unit tissue volume in the human forearm (33–35) than in the extremities of dogs or cats (36–38).

Proportionality between pressure head and flow is characteristic of the viscous flow of fluids through artificial porous membranes. From this point of view it will be interesting to determine if the filtration coefficient of the capillary membranes varies inversely with the viscosity at different temperatures as found with artificial porous membranes (39).

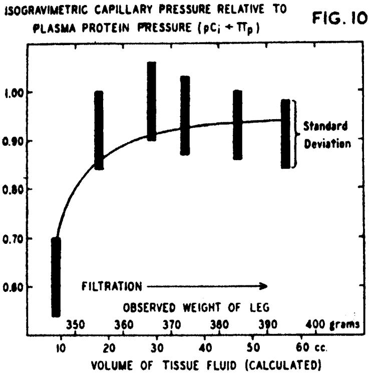
B. THE EFFECTS OF PROLONGED FILTRATION AND ABSORPTION. The results illustrated in figures 1 and 2 show that net filtration or absorption of small quantities of fluid (less than one gram per 100 grams tissue) occur at a constant rate following an increment or a decrement in mean capillary pressure. Evidence has been given by Krogh, Landis and Turner (40) and by Landis and Gibbon (32) that continued net filtration increases tissue pressure in the human forearm, thereby causing a progressive decrease in the rate of filtration following an increment in venous pressure. Continued net absorption, on the other hand, must eventually be limited by the volume and protein content of the fluid originally present in the tissue spaces, a limitation which was clearly envisioned by Starling (1) but which has never been evaluated experimentally.

Figure 10 shows the relations between the volume of tissue fluid and the total pressure opposing filtration (isogravimetric capillary pressure) in an isolated hindlimb of a dog. The design of the experiment was as follows. The tissue fluid was first concentrated by allowing net absorption to take place; capillary pressure was then increased above the isogravimetric value and 68 grams of fluid filtered from plasma to tissue fluid. The filtration process was interrupted at intervals in order to obtain isogravimetric values. Approximately five hours were required to complete the experiment.

It is seen that the isogravimetric capillary pressure obtained at minimum limb weight was only two-thirds of the plasma protein pressure. Presumably this is a result of the previous prolonged absorption with resulting concentration of protein in tissue fluid and diminution of effective osmotic pressure of plasma proteins. Filtration of 15 grams of fluid into the tissue spaces diluted the tissue fluid proteins by an amount sufficient to increase the isogravimetric pressure to 92 ± 8 per cent of the plasma protein pressure. Further filtration of 33 grams did not increase this value significantly and in this latter range of tissue fluid volumes the rate of filtration remained constant following an increment in capillary pressure.

In another experiment of this type filtration was continued until 175 grams of fluid had been filtered into the tissue spaces (65 per cent of the initial weight of the limb). Conditions were made such that the osmotic pressure of the proteins was only a small fraction of the total filtration pressure by diluting the plasma with a large volume of Ringer's solution and raising the mean capillary pressure to an abnormally high value. The results are summarized in table 4.

It is seen that the filtration rate remained constant until 90 grams of fluid had been filtered. The limb appeared moderately edematous at this time.



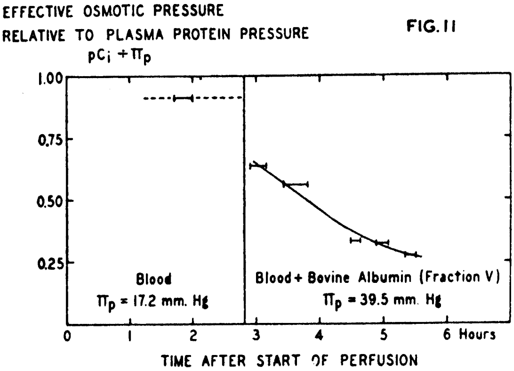


FIG. 10. THE EFFECTS OF PROLONGED FILTRATION IN PREVIOUSLY DEHYDRATED LIMB. The isogravimetric capillary pressure rises rapidly at small tissue fluid volumes but remains relatively constant over a wide range of higher tissue fluid volumes. Presumably this is the a result of dilution of protein in tissue fluid with capillary filtrate, which must contain protein in concentration less than that of tissue fluid at the end of the filtration period (0.5%). The smooth curve and the tissue fluid volume scale are calculated from the relation $V = (G_o + [P]_f \cdot \Delta W) \div [P]_t$ as explained in the text.

FIG. 11. THE EFFECTIVE PROTEIN OSMOTIC PRESSURE RELATIVE TO TRUE PROTEIN OSMOTIC PRESSURE following the addition of bovine albumin to the perfusing blood. Bovine albumin, unlike concentrated homologous plasma, is not retained by the capillary membranes in the hindlimbs of cats or dogs perfused under the conditions of these experiments.

Further filtration occurred at a progressively slower rate and this we attribute to developing tissue pressure for the protein pressure opposing filtration remained a small fraction of the mean capillary pressure. Evidently the isolated hindlimb differs from the human forearm in that tissue pressure does not become a significant factor opposing filtration until the limb is grossly edematous.

The results illustrated in figure 10 may be employed to estimate the volume of tissue fluid and the protein composition of the filtrate. Thus,

$$V = \frac{G_0 + [P]_f \Delta W}{[P]_t}$$

where V = volume of tissue fluid, G_o = total quantity of protein in tissue fluid at start of filtration, $[P]_f$ = concentration of protein in capillary filtrate, $[P]_t$ = concentration of protein in tissue fluid = $\psi(\pi_p - pC_i)$, ψ = relation between protein concentration and protein osmotic pressure given in table 1 and ΔW = increase in weight of limb during filtration.

This equation was employed to construct the 'tissue fluid volume' scale on the abscissa of figure 10. The protein concentration in capillary filtrate corresponding to the calculated curve is 0.3 per cent; this value best fits the observed data but other values, within the range 0.2–0.4 per cent, lie within the range of analytical errors.

DISCUSSION

Starling (1) considered the protein in tissue fluid to be an important factor in the regulation of the fluid exchange. To quote (p. 394): "With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid, until this becomes richer in proteids; and the difference between its (proteid) osmotic pressure and that of the intravascular plasma is equal to the diminished capillary pressure." A consideration of figure 10 shows that concentration of extravascular protein does in fact occur, but only after relatively large quantities of tissue fluid have been absorbed. In the intact animal the effective osmotic pressure would be diminished by dilution of the plasma proteins long before the concentration of protein in tissue fluid became a significant factor. It therefore appears likely that the principal osmotic factor regulating the fluid exchange normally involves changes in plasma protein concentration rather than changes in the protein concentration of extravascular fluid.

TABLE 4. PROLONGED FILTRATION AND EDEMA FORMATION
Mean capillary pressure 53 ± 3 mm. Hg. Protein pressure 9–13 mm. Hg. Initial weight of limb 270 grams

TIME	AMOUNT FILTERED	FILTRATION RATE GRAMS/MIN. IN 20-MINUTE PERIOD	VISIBLE EDEMA
minutes	*grams*		
0	0	—	None
20	45	2.25	+
40	90	2.25	++
60	126	1.80	+++
80	155	1.45	++++
100	171	0.80	++++

7. *Foreign protein.* In four experiments (three cats, one dog) bovine albumin was added to the perfusion reservoir. Two preparations of albumin were employed, *a*) Armour Co. 'Fraction *V*' which is said to contain about three per cent of impurities in the form of globulin fractions and acetate buffer; *b*) crystalline bovine albumin prepared by the Armour Co. The albumin was added in a 25 per cent solution titrated to pH 7.4. The results were similar for both preparations.

Figure 11 shows the effective osmotic pressure relative to plasma protein pressure before and after the administration of albumin. One hour after the addition of albumin the effective osmotic pressure across the capillary membrane was only 50 per cent of the protein pressure, a value which would obtain if the protein in tissue fluid were sufficient to exert an osmotic pressure of 20 mm. Hg (equivalent to 4.9 per cent albumin). Evidently purified or crystalline bovine albumin, unlike concentrated homologous plasma, is not retained by the capillary membranes in the hindlimbs of cats and dogs perfused under the conditions of these experiments. The reasons for this leakage are not clear; Heyl, Gibson and Janeway (41) found that crystalline bovine albumin is as effective as reconstituted human plasma in the restoration of blood volume following hemorrhage in humans.

Despite the large and progressive leakage of bovine albumin through the capillary membranes, the *filtration coefficient* remained unaltered. This is illustrated in figure 9 in which the net rate of fluid exchange for any given difference between mean capillary pressure and effective osmotic pressure was the same before and after albumin was added, although the effective osmotic pressure after the addition of albumin diminished progressively from 40 to 15 mm. Hg. The filtration coefficient was similarly unaffected by the leakage of protein which occurred at the end of each experiment as described in section 5 above. Evidently the permeability of the capillary wall to fluid is not necessarily related to the permeability to protein in the mammalian circulation.

SUMMARY

1. Methods are described for determining the following quantities in isolated perfused hindlimbs of cats and dogs.

a) Mean capillary pressure (accuracy ±0.5 mm. Hg),

b) Total pressure opposing net filtration of fluid from plasma to tissues (isogravimetric capillary pressure),

c) Net rate of fluid transfer across capillary membranes,

d) Resistance to blood flow from femoral artery to effective midpoint of capillary circulation and from capillary circulation to the femoral vein.

2. The rate of net fluid exchange between plasma and tissue spaces may be delicately adjusted over a wide range of values by varying the arterial pressure (fig. 2), the venous pressure (fig. 3) or the protein osmotic pressure of the plasma. At any given protein pressure there are an infinite number of pairs of values of arterial and venous pressures at which no net transfer of fluid occurs; two or more pairs of such values define the mean hydrostatic pressure in the capillaries (fig. 5).

3. The mean hydrostatic pressure in the capillaries at which no net transfer of fluid takes place (pC_i) is 93 ± 1.5 per cent of the normal osmotic pressure of the plasma proteins (π_p). In the range of plasma protein pressures, 8 to 32 mm. Hg, the relation is given by

$$pC_i = 0.95\pi_p - 0.56 \qquad \text{(fig. 8).}$$

Reasons are given for believing that pC_i is equal and opposite to the effective osmotic pressure of the plasma proteins.

4. The mean pressure head available for net fluid transfer across the capillary

membranes is the mean capillary pressure minus the isogravimetric capillary pressure ($pC - pC_i$). The rate of filtration or absorption is accurately proportional to this pressure head and is independent of the absolute protein or capillary pressures over a wide range of values (fig. 9). The proportionality constant is termed the 'filtration coefficient'; its mean value in the hindlimbs of 16 cats was 0.012 grams per minute per mm. Hg pressure difference across the capillary membrane per 100 grams tissue (table 3).

5. Absorption of fluid following a decrement in capillary pressure continues at a constant rate until the proteins in tissue fluid become sufficiently concentrated to oppose continued absorption. However, the quantity of protein present in tissue fluid is so small that a large volume of tissue fluid must be absorbed before the protein osmotic pressure of tissue fluid is significantly increased. It therefore appears likely that the principal osmotic factor regulating the fluid exchange normally involves changes in plasma protein concentration rather than changes in the composition of tissue fluid.

6. Filtration of fluid following an increment in capillary pressure continues at a constant rate until the limb becomes grossly edematous (table 4). In contrast to the intact human forearm, tissue pressure does not limit the rate of filtration over a wide range of tissue fluid volumes.

7. Crystalline bovine albumin, unlike concentrated homologous plasma, is not retained by the capillary membranes in the hindlimbs of cats or dogs perfused under the conditions of these experiments (fig. 11).

8. The resistance to blood flow from the femoral artery to the effective midpoint of the capillary circulation increases greatly when the blood flow is diminished by lowering the perfusion pressure (fig. 6). The effect is perhaps a result of anomalous viscous flow of blood in the arterioles; it is diminished or absent when the blood contains less than ten per cent red cells. The resistance to blood flow on the venous side of the capillary circulation is independent of flow even at normal corpuscular concentrations (figs. 5, 6 and 7). It is suggested that the dimensions of the venules are such as to minimize the effects of anomalous flow.

It is a pleasure to thank Prof. E. M. Landis, Prof. G. Scatchard, Dr. J. L. Oncley, Dr. S. W. Armstrong, Jr., Dr. D. M. Hegsted and Prof. C. A. Janeway for their help and suggestions in connection with these experiments. It is a pleasure also to acknowledge the technical assistance of Mr. Angelos Afentakis.

REFERENCES

1. Starling, E. H. Physiol. **19:** 312, 1896.
2. Landis, E. M. This Journal **82:** 217, 1927.
3. Landis, E. M. Physiol. Revs. **14:** 404, 1934.
4. Danielli, J. F. J. Physiol. **98:** 109, 1940.
5. Hyman, C. and R. Chambers. Endocrinology **32:** 310, 1943.
6. Palmer, G. H. and G. H. Joseph. This Journal **146:** 126, 1946.
7. Whittaker, S. R. F. and F. R. Winton. J. Physiol. **78:** 339, 1933.
8. Gaddum, J. H. J. Physiol. **67:** 16P, 1929.
9. Winton, F. R. J. Physiol. **87:** 65P, 1936.
10. Hepp, O. Z. ges. exptl. med. **99:** 709, 1936.

11. Brown, A. Private communication.
12. Scatchard, G., A. G. Batchelder and A. Brown. J. Clin. Invest. **23:** 459, 1944.
13. Scatchard, G., A. G. Batchelder and A. Brown. J. Am. Chem. Soc. **68:** 2320, 1946.
14. Cohn, E. J., J. L. Oncley, L. E. Strong, W. L. Hughes, Jr. and S. H. Armstrong, Jr. J. Clin. Invest. **23:** 417, 1944.
15. Scatchard, G. Personal communication.
16. Deutsch, H. F. and M. B. Goodloe. J. Biol. Chem. **161:** 1, 1945.
17. Adolph, E. F. and M. J. Leport. Proc. Soc. Exp. Biol. and Med. **28:** 963, 1931.
18. Scott, F. H., M. Rabinowitz and A. Rupp. Proc. Soc. Exp. Biol. and Med. **20:** 227, 1923.
19. Tani, I. Biochem. Z. **45:** 189, 1924.
20. Green, H. Personal communication.
21. Pappenheimer, J. R. and J. P. Maes. This Journal **137:** 187, 1942.
22. Green, H. D., R. Lewis, N. Nickerson and A. Heller. This Journal **141:** 518, 1944.
23. Bayliss, W. M. and E. H. Starling. J. Physiol. **16:** 159, 1894.
24. Luetscher, J. A., Jr. J. Clin. Invest. **20:** 99, 1941.
25. Perlman, G. E., W. Glenn and D. Kaufman. J. Clin. Invest. **22:** 627, 1945.
26. Landis, E. M., M. Jonas, M. Angevine and W. Erb. J. Clin. Invest. **11:** 717, 1932.
27. White, J. C., M. E. Field and C. K. Drinker. This Journal **103:** 34, 1933.
28. Field, M. E., O. C. Leigh, J. W. Heim and C. K. Drinker. This Journal **110:** 174, 1934.
29. Drinker, C. K. and M. E. Field. This Journal **97:** 32, 1931.
30. Drinker, C. K. and O. M. Yoffey. Lymphatics, Lymph and Lymphoid Tissue. Harvard University Press. pp. 61–65, 1941.
31. Drinker, C. K. Ann. N. Y. Acad. Sci. **46:** 807, 1946.
32. Landis, E. M. and J. H. Gibbon, Jr. J. Clin Invest. **12:** 105, 1933.
33. Grant, R. T. Clin. Sci. **3:** 157, 1937.
34. Allen, W. J., H. Barcroft and O. G. Edholm. J. Physiol. **105:** 225, 1946.
35. Harpuder, K., J. Byer and I. Stein. This Journal **150:** 181, 1947.
36. Burton-Opitz, R. This Journal **9:** 161, 1903.
37. Cammer, L. and F. R. Griffith. This Journal **125:** 699, 1939.
38. Kramer, K. and W. Quensel. Pflügers Arch. **239:** 620, 1937.
39. Duclaux, J. and J. Errera. Kolloid Z. **38:** 54, 1926.
40. Krogh, A., E. M. Landis and A. H. Turner. J. Clin Invest. **11:** 63, 1932.
41. Heyl, J., J. G. Gibson and C. A. Janeway. J. Clin. Invest. **22:** 763, 1943.

FORCE AND TIME ELEMENTS IN CIRCULATORY CHANGES UNDER ACCELERATION: CAROTID ARTERIAL PRESSURE DEFICIENCY AREAS[1]

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Acceleration, as developed by means of a centrifuge, has become a very useful tool in biological research. Large displacements of movable components of the living organism may be brought about by such a force transmitted to the organs and tissues. Several aspects of the effects of acceleration on the circulatory system have already been reported (1, 2). In the present study an evaluation of the relative effects on the arterial pressure of g force as a function of time has been made.

EXPERIMENTAL

Apparatus and methods have been previously described (2). The experiments were carried out on a centrifuge of 10-foot radius. Dogs and monkeys (*M. rhesus*) under local or general (usually amytal) anesthesia were used. Blood pressure in the carotid and femoral arteries was recorded by means of an electric resistance manometer. E.C.G., E.E.G. and respiratory rate were also commonly recorded; these served to determine various bodily changes and as a guide to the animal's condition. Most of the experimental runs were 5, 10, 15, 20, 25 and 30 seconds in duration at both positive (chiefly) and negative acceleratory forces of 1, 2, 3, 4 and (sometimes) 6 g. About 550 tests were carried out on dogs and about 450 on monkeys.

In earlier work on the circulatory changes which occur under exposure to high accelerations we considered among other features arterial pressure and flow relationships. Further useful and perhaps more significant measure of the circulatory disturbance may be made on the basis of the overall rise or fall in arterial pressure from the base level in time during and immediately after acceleratory test. Such a shift may be determined by measurement on a graph of the area circumscribed by the experimental blood pressure curve connected by a line drawn horizontally from the initial (basal) to the final blood pressure level at or shortly after the end of centrifugation. The circulatory change thus found may be described as the pt_p area, in which p represents arterial pressure change (mm. Hg), and t_p the time (seconds) from the first rise or fall in blood pressure until its return to normal. Determination of this area was accurately made with a planimeter. The acceleratory area (gt_g) was measured similarly (fig. 1).

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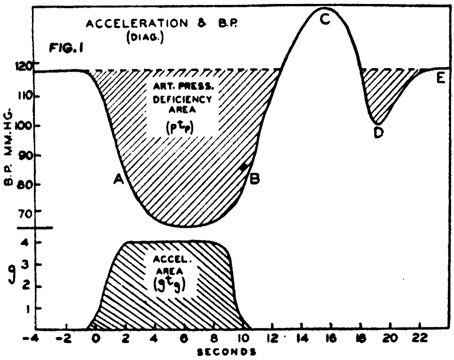
[1] Work carried out under a contract with the U. S. Navy, Office of Naval Research, and the University of Virginia.

An increase or decrease in arterial pressure under centrifugation may hardly be considered from the simple credit or debit viewpoint. It is nevertheless clear that reduction in blood pressure in arterial vessels supplying the head may constitute a serious functional loss or 'deficiency', and this term has been used in pertinent parts of this report. Emphasis has been made in a previous report of the significance of time and intensity factors in exposure to g forces (3).

RESULTS

Carotid arterial pressure deficiency. Average curves indicating the effect of various intensities and durations of g on the carotid arterial pressure of the monkey and dog are given in figures 2 and 3. The monkey showed a marked post-centrifugal increase in blood pressure above the normal level; it rose to a peak at about 15 seconds, and fell off thereafter. In the dog there was usually no similar rise (or only a slight one) in arterial pressure (fig. 3).



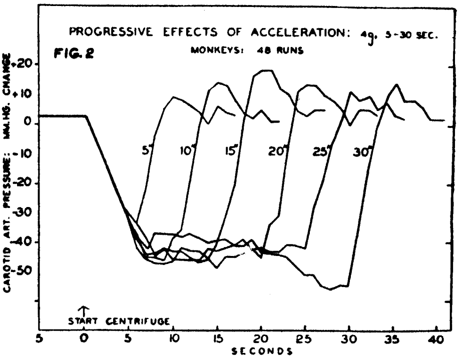


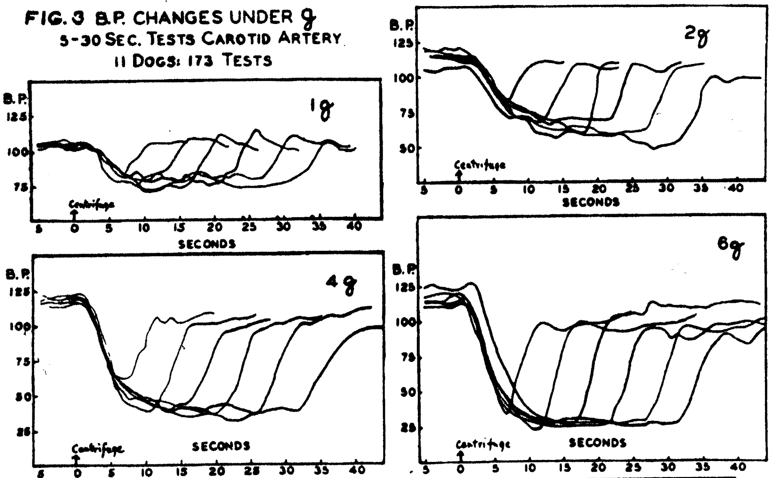
FIG. 1. GRAPH SHOWING COMPONENTS of carotid arterial pressure change during and after exposure to acceleratory forces (diagrammatic, monkey).

FIG. 2. CAROTID ARTERIAL PRESSURE CHANGES in the monkey during and after progressively increasing time exposures to 4 g. Arterial pressure levels, recorded continuously, are marked from second to second. Note post-acceleratory rises above the normal in blood pressure.

The data presented in table 1 demonstrate that for the dog the time required for the carotid arterial pressure to return to normal after centrifugation, at levels of 1, 2 and 4 g, was independent of the actual duration of the centrifuge test. However, $t_p - t_g$ was directly proportional to the magnitude of the acceleratory force to which the animal had been subjected. In the case of the monkey, the time interval $t_p - t_g$ was found to be practically independent of both duration and intensity of the exposure.

In figure 4 the pt_p areas for both animals tested were graphed against the corresponding gt_g areas. The results showed in each case a straight line relationship: that for the monkey was given by the equation pt_p area $= 11.5\ gt_g$ area, and for the dog by pt_p area $= 21.5\ gt_g$ area.

In the case of the dog a post-centrifugal fall in carotid arterial pressure was not generally observed following runs of 1 g; but at 2 and 4 g, post-acceleratory blood pressure deficiency areas were noted and measured (fig. 3; table 2). This secondary fall in arterial pressure seemed to be independent of the duration of the run, but increased directly with the intensity of g.



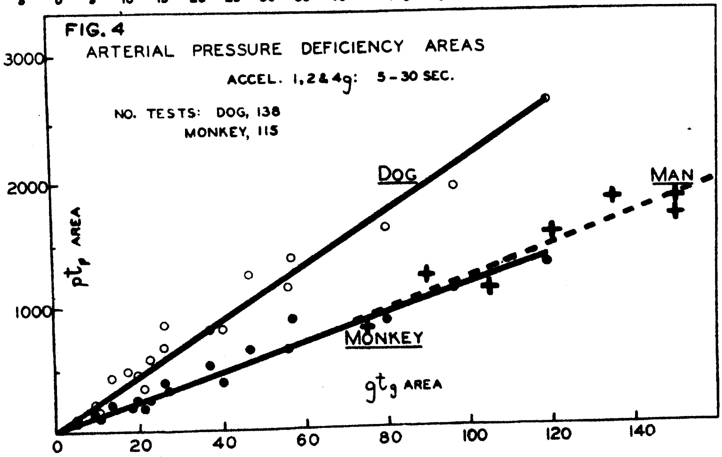


FIG. 3. CAROTID ARTERIAL PRESSURE CHANGES in the dog under different times and intensities of g. No post-acceleratory rises in blood pressure above normal occur in the dog, in contrast to the monkey (fig. 2).

FIG. 4. ARTERIAL PRESSURE DEFICIENCY AREAS compared in monkey and dog. Under given conditions, far greater circulatory shifts occur in the dog than in the monkey (see text).

The above observations led to the investigation of the $pt_p:gt_g$ relationship from a somewhat different point of view. Four dogs were exposed in 43 experiments to different g forces for varying periods of time, so that the product of g and t_g in all cases equalled 48 g-sec. Carotid arterial pressure curves were secured

TABLE 1. Carotid arterial pressure deficiency areas under acceleration

Animals subjected to various intensities and durations of g

No. of tests: monkey, 115; dog, 138

ACCELERATION	ACTUAL TIME OF EXPOSURE	TIME FROM INITIAL DROP OF B.P. TO ITS RETURN TO NORMAL		ACCELERATORY AREAS	$t_p - t_g$		ARTERIAL PRESSURE DEFICIENCY AREAS	
		Dog	Monkey		Dog	Monkey	Dog	Monkey
g	t_g sec.	sec.	t_g sec.	gt_g			pt_p	pt_p
1	7.4	9.0	8.7	5.0	1.6	1.3	101	76
1	13.1	13.5	14.0	9.6	0.4	0.9	223	173
1	17.3	18.5	17.6	13.5	1.2	0.3	423	290
1	22.1	23.5	23.0	18.2	1.4	0.9	478	233
1	28.0	28.0	27.0	22.5	0.0	−1.0	573	304
1	32.5	33.0	33.3	26.9	0.5	0.8	844	337
2	7.8	12.5	6.9	10.2	4.7	−0.9	171	131
2	13.1	21.5	12.2	19.5	8.4	−0.9	502	233
2	18.0	21.5	18.0	26.0	3.5	0.0	688	348
2	23.0	31.0	23.5	37.0	8.0	0.5	878	583
2	28.0	34.0	26.7	46.5	6.0	−1.3	1,290	633
2	33.1	37.5	35.0	57.0	4.4	1.9	1,420	839
4	7.4	19.0	9.4	21.0	11.6	2.0	456	234
4	12.7	25.0	14.8	40.0	12.3	2.1	938	475
4	17.2	25.5	18.0	56.0	8.3	0.8	1,270	785
4	23.0	34.5	25.2	80.0	11.5	2.2	1,760	1,020
4	28.0	40.5	32.8	96.7	12.5	4.8	2,070	1,350
4	34.0	51.0	36.1	119.0	17.0	2.1	2,790	1,530

TABLE 2. Post-acceleratory carotid arterial pressure deficiency areas

Dog: 138 runs

g	TIME OF EXPOSURE	POST-CENTRIFUGAL A.P. DEFICIENCY AREAS	g	TIME OF EXPOSURE	POST-CENTRIFUGAL A.P. DEFICIENCY AREAS
	sec.	pt_p		sec.	pt_p
1	5–30	0	4	5	108
2	5	(3)	4	10	134
2	10	39	4	15	128
2	15	16	4	20	188
2	20	73	4	25	128
2	25	33	4	30	186
2	30	43			

TABLE 3. Acceleration and carotid arterial pressure deficiency areas

$g \times t_g$ = constant; dog: 43 tests

g	TIME OF EXPOSURE	$g \times t_g$	A.P. DEFICIENCY AREAS
	sec.		pt_p
3	16	48	1,065
4	12	48	1,054
5	9.5	48	1,059
6	8	48	1,135

TABLE 4. COMPARISON OF ARTERIAL PRESSURE AREAL CHANGES IN DOG AND MONKEY UNDER POSITIVE AND NEGATIVE g FORCES

Exposure time, 10 sec.

ACCELERATION	ANIMAL	NO. TESTS	ARTERIAL PRESSURE AREA CHANGE		CAROTID-FEMORAL DIFFERENCE
			Carotid Artery	Femoral Artery	
g			pt_p	pt_p	
+1	Dog		−128	+ 68	−60
+2	“	178	−274	+242	−32
+3	“		−438	+358	−80
+1	Monkey		− 92	+272	+180
+2	“	118	−191	+345	+154
+3	“		−378	+542	+164
−1	Dog		+ 84	−200	−116
−2	“	162	+126	−382	−256
−3	“		+406	−512	−106
−1	Monkey		+208	− 66	+142
−2	“	183	+349	−142	+207
−3	“		+578	−265	+313

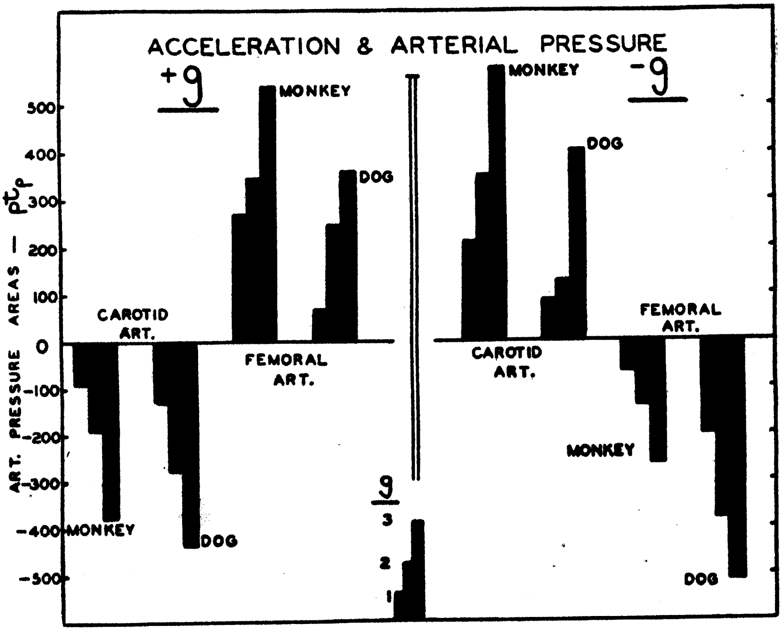


FIG. 5. CHANGES IN CAROTID AND FEMORAL ARTERIAL PRESSURES (areas) in the dog and monkey under exposure to positive and negative accelerations.

and the pt_p deficiency areas calculated. The study revealed clearly that for a given gt_g value, the resultant pt_p areas were a constant (table 3).

Positive and negative g effects compared. An analysis of arterial pressure areal changes shown by animals under positive and negative g forces also indicated striking differences in response of the monkey and dog. Overall changes in the carotid and femoral arterial pressures have been reported in these cases (4). The arterial pressure areal changes have now been determined from over 600 tests, and the data are shown in table 4.

It is striking that in the case of the monkey compared to the dog there is less fall in the carotid and greater rise in the femoral pressure under positive g, and greater rise in the carotid and less fall in the femoral under negative g forces. In comparison with the overall arterial pressure deficiency observed in the dog, the monkey shows a considerable 'credit balance' in arterial pressure under both positive and negative accelerations. Further, and perhaps more important, circulation through the cerebral tissues appears to be better maintained in the anthropoid animal under stress than in the case of the dog (table 4, fig. 5).

DISCUSSION

In animals exposed to acceleratory forces, carotid arterial pressure follows a pattern which is expressed diagrammatically in figure 1, derived from several hundred experiments. After the main fall in arterial pressure (A), there occurred toward the end of the exposure a rather rapid increase in level (B), until a post-centrifugal pressure peak (C) was reached which was often higher than the initial or basal level. This peak was followed by a fall in arterial pressure (D) below the initial pre-test value, then a gradual climb toward the normal level (E). The post-centrifugal fall in arterial pressure may be a reflection of the transient functional disturbance caused by exposure to g forces.

In the dog the time interval $t_p - t_g$ (table 1) was dependent on the intensity of g; in the case of the monkey, on the other hand, return to normal pressure levels following centrifugation was practically independent of the intensity of the exposure up to 4 g. This observation is probably of importance in differentiating between the effect of acceleratory forces on quadruped (dog) and upright (monkey) mammalian forms. The monkey and man as well, perhaps through vascular adjustments (reflexes, etc.) developed in the course of evolution to support the upright posture, are more resistant than lower forms to gravitational forces which may tend seriously to reduce blood pressure in the head end of the animal.

Data on the reactions of man on the centrifuge have been derived from figures published by Wood *et al.* (5) and are included in figure 4 herewith[2]. It is clear that the resistance of man to acceleratory forces is equally as good as that shown by *Macacus rhesus*, on the basis at least of arterial pressure reduction in the carotid vessel.

It is essential to consider the various factors involved in relation to one another rather than separately. Two of the variables, g and t_g, are independent; the others, degree and duration of blood pressure reduction, are dependent. It is the area gt_g which determines the area of arterial pressure change, pt_p; i.e., it does not matter how, for example, 100 gt_g units have been produced—they may have been produced by a short exposure to high g or a longer exposure to lower g—provided the gt_g areas are the same (within limits), the resultant pt_p areas will be similar. For this reason the simple minimal level of blood pressure alone does not fully express the physiological difficulty during and responses after centrifugation. Earlier work from this laboratory also suggests that mortality following exposure to centrifugation is a direct function of the product of time and intensity of exposure to g (1).

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[2] Acknowledgement is made of the permission of the Mayo Aeromedical Unit to compare these results.

SUMMARY

The reduction in carotid arterial pressure observed during exposure to acceleratory forces (monkey, dog; 1–4 g, 5–30 sec.) may be considered as a blood pressure deficiency area (pt_p). Thus, one may determine the carotid arterial pressure deficiency as a function of time. This area was found to be directly proportional to the intensity and duration of centrifugation (gt_g), and for a given acceleratory area the circulatory deficiency area was a constant.

Monkeys were found to be much more resistant to acceleration than dogs. In the monkey the arterial pressure deficiency areas on exposure to 1, 2 and 4 g were only about half as great as those in the dog; further, the recovery areas (rebound) were marked in the former and usually absent in the latter case. Carotid arterial pressure reactions in man are similar to those of the *Macaque*.

A comparison of dog and monkey responses under positive and negative g forces also indicated that circulation through the cerebral tissues was maintained better in the anthropoid type. The differences in response may be referable to the different stance and thus the reflex development of the two animal types.

REFERENCES

1. Britton, S. W., E. L. Corey and G. A. Stewart. This Journal **146:** 33, 1946.
2. Britton, S. W., V. A. Pertzoff, C. R. French and R. F. Kline. This Journal **150:** 1, 1947.
3. Britton, S. W. Report No. 1, O.S.R.D., C.M.R., Washington, 1942.
4. Britton, S. W. This Journal, 1948. In press.
5. Wood, E. H., E. H. Lambert, E. J. Baldes and C. F. Code. Federation Proc. **5:** 327, 1946.

EFFECT OF SYMPATHECTOMY ON BLOOD FLOW IN THE HUMAN LIMB

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Clinical papers (1, 2) testify to the increasing frequency with which sympathetic block and resection are being used in the treatment of the peripheral vascular diseases, particularly those in which intermittent claudication is the chief manifestation.

Herrick, Essex and Baldes (3) showed that the blood flow in the femoral artery in the sympathectomized limb of the dog was approximately double that of the normal side. They measured total blood flow which does not distinguish between the circulatory requirements of skin and muscle. Grant and Pearson (4) pointed out the difference in behavior of the circulation in the distal and proximal parts of a human limb. The former, a measure of the skin circulation, responds to sensory stimuli and to adrenalin by vasoconstriction; the latter, the bulk of which is composed of skeletal muscle, responds to sensory stimuli not at all and to adrenalin by vasodilatation. They also showed that there was no difference in the hyperemia of exercise in a forearm deprived of its sympathetic innervation and its normal mate. Since then others, notably Wilkins and Eichna (5), have commented upon the lack of effect of sympathectomy upon muscle blood flow and have even intimated that such an operation might be harmful because vasodilator fibers also are sectioned. This part of their argument presupposes that such a system of nerve fibers actually exists.

Our approach in clarifying the problem is based on a comparison of the blood flow in sympathectomized and normally innervated human limbs.

METHOD

Blood flow was measured by the venous occlusion method (6) which although indirect is reliable and readily applicable to the unanesthetized human. A water plethysmograph of the Abramson type (7) was used for the calf; for the foot, the apparatus described by Kunkel and Stead (8) was more satisfactory.

Eleven of the 12 patients comprising this report had vascular disease in the lower limbs of varying degree and etiology. The remaining individual had persistent hypertension for which the Smithwick operation was done. The highlights of their clinical histories are incorporated in the tables.

The interval between operation and our studies was as short as a week in some instances, while in others it was from six months to several years. In four acute experiments, the test limb also served as control, inasmuch as comparable blood flow records were taken before and after operation. In the others, the contralateral limb served as control or the 'normal' for the individual. Such a comparison was felt to be valid as intensive clinical examination of the peripheral vessels disclosed no striking difference between the two limbs.

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[1] Aided by a grant from the Sigmund M. Lehman Fund at the Montefiore Hospital, New York City, and the Martha Hall Foundation.

Although the segmental distribution of the autonomic nervous system is still incompletely known in some respects, interruption of the ganglia at the level of L2 and L3 has been shown to produce effect from the level of the knee down (9), including the calf muscles. As a check on the adequacy of operation, determination of the rise in skin temperature and of changes in the electrical resistance of the skin was made. These tests as well as the histological report on material removed at operation enabled us to state unequivocally that a sympathectomy had been done, that it was complete and that it included the test region.

Tests were run with the patient seated comfortably in a chair with the leg outstretched and parallel to the ground. When blood flow to the calf was measured, the circulation to the foot was eliminated by a tourniquet applied just distal to the plethysmograph.

An indifferent water temperature of 34 to 35°C. was maintained on the enclosed segment of limb for 30 minutes. The room temperature was constant for each experiment but varied between 23°C. maintained during the winter season and 30°C. during the summer. This is of little importance for circulation of the calf in which we were especially interested. We have confirmed Grant's observation (4) that the blood flow of resting muscle is remarkably constant even though there are such variations in the environmental temperature. However, in the case of the hand or foot, even small temperature changes may profoundly affect the blood supply.

Blood flows of the arbitrarily defined skin and muscle circulation were taken in the resting state described above. This was the average of several test runs which as a rule differed so little that the inflow curves were almost parallel. It was felt that only variation of 20 per cent or more in successive blood flow determinations could be regarded as significant.

Measurements were also taken during the phase of reactive hyperemia. Upon occluding the arterial circulation for ten minutes and then releasing it, a profound increase in blood flow as well as amplitude of the pulse wave was registered on the tracing. The characteristic flush was easily visible in the portions of the extremity outside the plethysmograph. It is our belief that the blood flow during reactive hyperemia is an index of the available peripheral circulation.

In certain instances blood flow estimations were made with the limb segment exposed to water of 45°C. and after exercise of the calf muscles consisting of 50 alternate flexions and extensions at the ankle.

RESULTS

1. *Blood flow in the foot* (*table 1*). The skin of a recently sympathectomized limb is warm, dry and bright in color. Although these findings tend to persist, there is sometimes a return of skin temperature to preoperative levels in a matter of weeks. Loss of sweating may then be the sole objective sign of a technically complete denervation and may also be partly responsible for making the sympathectomized limb feel warm.

With the rise in skin temperature there is an increase in blood flow. Its magnitude is dependent on two factors: the available vascular bed and the level of the resting blood flow prior to operation.

The maximum increase in blood flow on the sixth day and again three weeks after operation is only 10 per cent in P. Ga., age 21 years, whose basic disease was thrombo-angitis obliterans. This is well within the limit of error. It must be mentioned that this patient had a gangrenous ulcer on the little toe of the test foot with an inflammatory response in the surrounding tissue, so that vasodilatation was almost complete before operation. Thus the resting blood flow preoperatively approximates the flow during the phase of reactive hyperemia and this accounts for the negligible increase.

In J. R., age 36, who also had Buerger's disease, the blood flow in the test foot increased 85 per cent over its preoperative level on the eighth day.

TABLE 1. BLOOD FLOW IN FOOT (cc/min/100 grams of tissue)

SUBJECT	AGE	RESTING BLOOD FLOW		REACTIVE HYPEREMIA (BLOOD FLOW)	% CHANGE AFTER SYMPATHECTOMY
		Control	Sympathectomy		
P. Ga.[1]	21	5.4	1) 5.6 2) 6.3	7.6	+ 10
J. R.[1]	36	3.5	6.4	8.0	+ 85
R. M.[1]	38	3.8	5.6	10.0	+ 45
J. K.[1]	56	2.8	6.0	20.0	+115
C. J.	45	3.0	5.9	10.0	+ 95

[1] Same foot used.

Patient R. M., age 38, had essential hypertension. Two weeks after the first stage of the Smithwick procedure, the increase is only 45 per cent in spite of the fact that her peripheral vessels were clinically normal. It is known that the extensive circulatory readjustments that follow such a procedure militate against marked vasodilatation in the extremities.

Our fourth patient, J. K., age 60 years, had a history of cold injury which resulted in amputation of several toes for gangrene. His feet, which were extremely cold and sweaty prior to operation, became warm and dry. The increase in blood flow ten days after sympathectomy is 115 per cent.

Patient C. J., age 45 years, illustrates the long-term effects of sympathectomy in the foot. In this individual, where the underlying disease was again thrombo-angitis obliterans, the blood flow in the sympathectomized foot six months after operation is 95 per cent greater than in the 'control' foot.

Inasmuch as sympathectomy for peripheral vascular disease was seldom performed unless there was an adequate rise of skin temperature after paravertebral novocaine block, this automatically excluded from our series most patients with a markedly deficient vascular supply.

2. *Blood flow in the calf.* The resting blood flow of a muscular region like the calf, as determined by the technique employed, ranges from 1.5 to 3.0 cc. per minute per 100 grams of tissue but is fairly constant for the same individual. It has been calculated by Grant (4) that the intense cutaneous hyperemia produced by multiple puncture of histamine into the skin results in an increase of

one to two cc. of blood flow per minute per 100 grams of tissue. This figure represents the increase due to complete vasodilatation in the skin and only measures which produce more than this, which is roughly an increase of 100 per cent, can be considered as affecting muscle blood flow.

It is quite striking, therefore, that in the 12 patients in whom the sympathetic outflow was resected there was no such significant increase.

In contrast, the increase in blood flow during the phase of reactive hyperemia may be as much as 300 to 1200 per cent of the resting value. Less marked but also significant is the increase which results from moderate exercise of the calf muscles or from heating the segment of calf enclosed in the plethysmograph.

TABLE 2. Blood flow in calf (cc/min/100 grams of tissue)

SUBJECT	AGE	DISEASE	TIME SINCE OPERATION	RESTING BLOOD FLOW		REACTIVE HYPEREMIA (BLOOD FLOW)	% CHANGE AFTER SYMPATHECTOMY
				Control	Sympathectomy		
P. Ga.[1]	21	TAO[2]	1) 6 days 2) 21 days	2.7	1) 2.1 2) 2.3	19	−20
J. R.[1]	36	TAO	8 days	2.1	1.6	15	−25
R. M.[1]	38	Essential hypertension	14 days	1.9	1.9	10	0
J. K.[1]	56	Cold injury	8 days	2.7	3.5	18	+30
C. J.	45	TAO	6 months	1.5	2.9	13	+93
J. McI.	60	Cold injury AS[3]	15 days	1.2	1.2	8.2	0
T. N.	63	AS	1 year	Bilateral lumbar sympathectomy	Left) 2.2 Right) 2.1	2.3 4.5	—
E. B.	60	AS	14 days	2.6	3.7	9.5	+40
P. Gl.	38	TAO	4 months	2.3	2.3	14	0
F. C.	44	Raynaud's syndrome	2½ years	Quadrilateral sympathectomy	2.8	36	—
L. G.	33	Diffuse vascular disease	3 years	Bilateral lumbar sympathectomy	3.4	10	—
W. F.	67	AS	15 days	Unilateral amputation	2.9	11.2	—

[1] Same calf used. [2] Thromboangiitis obliterans. [3] Arteriosclerosis.

The increase in blood flow to any of these measures is limited by the available vascular bed. Thus, in a younger patient like P. Ga., this potential reservoir is much greater than in an older individual, T. N., whose pain at rest indicates that the tissues are insufficiently supplied with blood even under basal condition.

DISCUSSION

This study shows that sympathetic denervation of the human limb results in an increased blood flow in areas where skin predominates but leaves the blood flow in muscular regions relatively unchanged. Any augmentation noted in the latter can easily be explained by the coexisting cutaneous vasodilatation. Even in sympathectomized regions where skin circulation predominates, the relation of resting flow to the blood flow during reactive hyperemia shows that maximal vasodilatation is not present within a week after operation.

In man, vasoconstriction of the skin, an increase in pilomotor and sudomotor activity and vascular dilatation in muscle are the proven effects of sympathetic stimulation and adrenalin discharge on the limbs (4, 10, 11). Conversely, resection of the sympathetic pathways leads to cutaneous vasodilatation and loss of sweating and hair erection in the involved region. Our results bear out the contention arrived at from the intra-arterial injection of adrenalin: that muscle vessels would not be dilated by the removal of sympathetic control.

The vascular bed of a muscular region can be opened wide by exercise, by direct heating of the limb and by creating a blood deficit through occlusion of the main artery. Each of these measures produces a significant increase in blood flow through the release of vasodilating metabolites. Similar increases take place despite the presence of definite vascular disease, provided the collateral circulation is adequate. The constant values for blood flow in muscle after sympathectomy permit the conclusion that innervation of blood vessels is not the important factor in regulating the circulation in muscular tissue. This favors for skeletal muscle an assumption which has been previously made for the brain (12) and cardiac muscle (13); namely, that it is the local demand which decides the blood flow to the active area.

Comparing the vascular reactions of blood vessels in normal muscle with those in the muscle of patients with vascular disease may seem unwarranted. There is no proof, however, that there is an essential difference in their behavior to sympathectomy. The circulation in areas where skin predominates, such as the hand and foot, is increased after sympathectomy both in the normal state and in the peripheral arterial diseases. Such an increase is quantitatively less striking in the latter, because of a diminution of the vascular bed through organic obstruction of blood vessels and sometimes because maximal vasodilation is reached as a result of secondary inflammation or due to compensatory circulation developed in response to the local ischemia.

From a clinical viewpoint, important inferences may be drawn. 1) Exercise, direct heating or anoxia of tissue increase muscle blood flow, but a sympathectomy is relatively impotent in this respect. The first two procedures actively increase the metabolism of the limb, while in the third the blood deficit is rapidly repaid on release. Each of these modalities, somewhat modified, is used in the treatment of peripheral vascular disease (reflex vasodilatation, intermittent venous occlusion); each has practical disadvantages which curtail its use. The ideal measure is one which would cause vasodilatation without increasing the metabolism of ischemic tissues.

2. Resection of the sympathetic trunk in an attempt to increase blood flow and thereby reduce the pain of muscular ischemia, or to increase the functional capacity for exercise or as a prophylactic against the reduction of muscle circulation by the occlusive vascular diseases, appears to be of doubtful value.

3. The use of sympathectomy for effect on the skin or lesion of the hand or foot is logical and sound physiologically. Thus in hyperhidrosis of severe degree, in disfigurement due to marmorization of the skin, and more frequently in Raynaud's phenomena and in the causalgic state, there is such marked relief both subjectively and objectively that the procedure seems justified.

tions which have been intractable to other treatment frequently heal. On occasions we have seen beginning gangrenous lesions of the toes slough and clear up promptly following sympathectomy. We have also seen toes which merely appeared poorly nourished and cyanotic become gangrenous immediately after operation. It is reasonable to assume that blood had been diverted from the local area which needed it desperately to a large skin surface with resultant detrimental effect on the area for whose benefit the sympathectomy was originally planned.

Furthermore, there are large gaps in our knowledge of the autonomic nervous system, not only as regards function but also anatomical distribution. Some evidence is accumulating for the existence of vasodilator fibers within the sympathetic system in man (14). If the sympathetic trunk is severed, such fibers which might play a beneficial rôle are removed with the vasoconstrictor fibers. On the other hand, if the sympathetic pathways carry afferent impulses, their interruption might very well be warranted for relief of pain, whether due to intermittent claudication or other factors. A recent article on afferent conduction by Threadgill merits attention in this respect (15). However, we should not at the present time base the need for operation on these hypotheses but on the more solid foundation of effect on muscle or skin vessels.

SUMMARY

1. A lumbar sympathectomy which is effective when gauged by the changes produced in an area of predominant skin circulation (foot) produces minimal increase in the circulation of a predominantly muscular area (calf).
2. The effective stimulus in increasing muscle blood flow is one which releases vasodilating metabolites. Exercise, direct tissue heating and arterial occlusion and release are examples.
3. The clinical application of sympathectomy for lesions or symptoms in areas primarily of skin circulation and other areas which are composed chiefly of muscle are discussed. The use of sympathectomy for the latter is of doubtful value.

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REFERENCES

1. Freeman, N. E. and H. Montgomery. Am. Heart J. **23:** 224, 1942.
2. Gage, M. Surgery **7:** 792, 1940.
3. Herrick, J. F., H. F. Essex and E. J. Baldes. This Journal **101:** 213, 1932.
4. Grant, R. T. and R. S. B. Pearson. Clin. Science **3:** 119, 1938.
5. Wilkins, R. W. and L. W. Eichna. Bull. Johns Hopkins **68:** 425, 1941.
6. Hewlett, A. W. and J. G. Van Zwaluwenberg. Heart **1:** 87, 1909, 1910.
7. Abramson, D. I. Vascular Responses in Extremities of Man. Chicago: University of Chicago Press, 1944.
8. Stead, E. A., Jr. and P. Kunkel. J. Clin. Invest. **17:** 711, 1938.
9. White, J. C. and R. H. Smithwick. Autonomic Nervous System. 2nd ed. New York. Macmillan Co., 1941. P. 425.
10. Harpuder, K., J. Byer and I. D. Stein. This Journal **150:** 181, 1947.
11. Allen, W. J., H. Barcroft and O. G. Edholm. J. Physiol. **105:** 255, 1946.
12. Schmidt, C. F., S. S. Katy and H. H. Pennes. This Journal **143:** 33, 1945.
13. Shipley, R. E. and D. E. Gregg. This Journal **143:** 396, 1945.
14. Burns, J. H. Physiol. Revs. **18:** 137, 1938.
15. Threadgill, F. D. Surgery **21:** 569, 1947.

EFFECTS OF EXERCISE AND OF SOME OTHER INFLUENCES ON THE RENAL CIRCULATION IN MAN[1]

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Relatively few quantitative reports are available on reflex influences on the renal circulation in man. Smith (1, 2) found that both diodrast and inulin clearances fell on changing from recumbent to upright or near upright position; filtration fraction showed slight rise or no change. McCann and Romansky (3) found that on changing from recumbent to erect position patients with nephroptosis showed falls in diodrast clearance with no significant change in inulin clearance, the filtration fraction rising; normal subjects and hypertensives without nephroptosis showed no significant postural changes in clearances or in filtration fraction. Brun, Knudsen and Raaschou (4) confirmed the findings of many earlier workers that urine flow is lower in the erect (or 60° vertical) position than in recumbency. Inulin clearance falls in the vertical position, with an increase in the percentage of filtered water reabsorbed. Diodrast clearance falls more than inulin clearance, giving a rise in filtration fraction. If the erect postion is maintained to the occurrence of syncope, diodrast and inulin clearances and urine flow fall abruptly (1, 2, 5); there is evidence that increased secretion of pituitrin may contribute to the postsyncopal oliguria (5).

Smith (1, 2) found a marked fall in diodrast clearance with marked rise in filtration fraction when subjects became alarmed. Wolf (6) found that a mildly painful cold stimulus (immersion of one hand in water at 5–10° C.) increased the diodrast and inulin clearances, with no change in filtration fraction; in the post-stimulus period diodrast clearance usually fell with no decrease in inulin clearance, giving a great increase in filtration fraction. More severe pain, by pressure on head, reduced both diodrast and inulin clearances, with rise in filtration fraction. There is great variability in Wolf's findings.

Diodrast clearance decreased by 18 to 54 per cent as a result of running 440 yards at maximum speed, and remained below the pre-exercise level for 10 to 40 minutes after exercise. Inulin clearance fall was less than diodrast clearance fall and might not occur. Exercise for 15 minutes on a bicycle ergometer also reduced clearances. There was no correlation between changes in clearances and in urine flow (7) and no mention is made of proteinuria.

This paper reports on the effects of postural changes, of a cold stimulus, of exercise, and of increased protein intake on para-aminohippurate (PAH) and inulin plasma clearances and, in some experiments, on plasma glucose levels, on occurrence of proteinuria, on blood pressure and on renal vascular resistance.

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[1] Aided by a grant from the Commonwealth Fund.

Urine volumes were also followed, but water intake was not closely enough controlled to permit other than gross conclusions as to the effects on urine flow. The subjects were healthy males, all except one (*HLW*, age 51) being medical students in the age group 20 to 30 years.

METHODS

Except for the experiments with high protein intake, the subjects had a light breakfast and omitted lunch on the days of experiments, which began about 2:00 P.M. Urine flow was promoted by drinking one to two liters of water during the 60 to 90 minutes before the beginning of the first clearance period. The subjects were recumbent for 30 to 40 minutes before the clearance periods began, the first two of which were controls in recumbency. Clearance periods, except for some shorter periods of running, were about 20 minutes. A priming intravenous dose of 19 mgm. inulin per kilo was followed by a sustaining subcutaneous dose of 90 mgm. inulin and 30 mgm. PAH per kilo. The intravenous inulin was the 10 per cent solution of U. S. Standard Products Co., Woodworth, Wis. The subcutaneous injection was prepared by boiling the necessary amount (0.9 cc. per kilo) of the 10 per cent inulin to about one-third its original volume and adding the 20 per cent PAH solution[2] (0.15 cc. per kilo). Thus, an 80 kilogram subject received 15.2 cc. of 10 per cent inulin intravenously, followed by a mixture of 24 cc. of 30 per cent inulin and 12 cc. of 20 per cent PAH subcutaneously. The 20 per cent PAH is too irritating to be injected undiluted subcutaneously. The subcutaneous injection was given in two or three postaxillary sites and the needle moved two or three times for each skin puncture, so that only a few cc. were given in one region. No anesthetic was used. The first clearance period was begun 20 to 25 minutes after ending the subcutaneous injection. This method obviated the necessity of continuous intravenous infusion and permitted the subject to move about freely for any desired experimental procedure (8). A few representative plasma level curves are given in figure 1; mean plasma levels during the respective clearance periods are calculated from such curves.

Urine collections were by voluntary emptying of the bladder. Some days prior to an experiment the subject obtained a water diuresis curve; smoothness of the curve was taken to indicate satisfactory sample collections. We are convinced that the majority of normal, intelligent, co-operative human males can quickly learn to empty the bladder quantitatively, and that a smooth water diuresis curve is a good index of such ability. Blood samples were heparinized and centrifuged immediately after collection. Blood pressures were obtained frequently by the auscultatory method; mean pressure was taken as diastolic plus 40 per cent of pulse pressure and the values given in the tables are the averages of values during a corresponding clearance period. Renal resistance units per square meter (RRU/M^2) are expressed as

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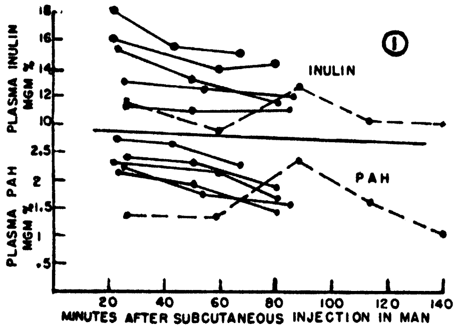
[2] The PAH solution was generously furnished by Sharpe and Dohme Co., Philadelphia, Pa.

$$\frac{\text{mean arterial pressure (mm. Hg) minus 5.}}{\text{renal blood flow per sec. per M}^2}$$

Renal blood flow was taken as twice the PAH plasma clearance; errors due to hematocrit variations were neglected, since we were interested only in the grosser directional changes in renal vascular resistance. Filtration fraction (FF) is $\frac{\text{inulin clearance.}}{\text{PAH clearance}}$ The zero settings of the photoelectric colorimeter for the plasma PAH and the plasma inulin determinations were made on pre-injection blank plasma filtrates; correction was made for changes in color production in the plasma inulin procedure due to changes in plasma glucose.



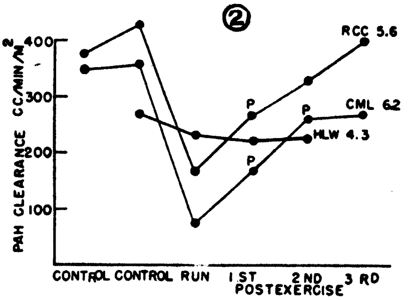


Fig. 1. Inulin and PAH plasma levels following intravenous priming and subcutaneous sustaining injections used in these experiments. The solid line separates the inulin from the PAH curves. The broken curves are from an exercise experiment; the first two points are during rest, while the third points are higher because of the diminished rate of excretion during exercise between second and third samples.

Fig. 2. PAH clearances before, during and after exercise on subjects, *HLW*, *RCC*, and *CML*. Speed of running in miles per hour at ends of curves. Presence of protein in urine samples is indicated by P.

Effect of high protein intake. Control periods in recumbency six hours after a light breakfast were obtained in two subjects on uncontrolled diet containing about 90 grams of protein daily. Some days later clearances were again obtained in recumbency four hours after a large protein meal, consisting largely of beef steak. On one subject, HW, clearances were again obtained after a week of high protein diet (220 grams of protein with 3400 calories daily). The results are seen in table 1. Increase in protein intake as a single meal had no effect on PAH clearance or on renal vascular resistance but slightly raised inulin clearance; high protein intake for a week raised PAH clearance 18 per cent and inulin clearance 31 per cent, and lowered renal vascular resistance 12 per cent.

Postural effects. Five experiments on the effects of postural changes were carried out on five subjects; the results are seen in table 2. The PAH clearance in the standing period was 12, 24, 15 and 23 per cent lower than the average of the two lying periods for the first four experiments of table 2, averaging 19 per cent lower. The inulin clearance in the standing period was 1, 18, 8 and 6 per cent lower than the average of the two lying periods, averaging 8 per cent lower. The inulin clearance fall on standing may be compared with an earlier finding (9) that endogenous creatinine output is 9 per cent lower on standing than in recumbency. In the earlier work clearances cannot be calculated since no plasma determinations were made but, since the plasma creatinine levels remained constant, the rates of urinary output are proportional to the clearances. Subject RO showed some vasomotor disturbance at the time of the subcutaneous injection, losing consciousness for a brief period. It is believed that the clearance values of his first period are abnormally low, due to renal vasoconstriction as a result of the psychic reaction to the injection; the second control period should be taken as the recumbent control with which to compare the standing period. When this is done there is seen on standing a fall of 23 per cent in PAH and a rise of 10 per cent in inulin clearance, the filtration fraction rising from 0.162 to 0.231. Thus in all cases the filtration fraction was higher during standing, the inulin clearance usually showing a slight fall but less than the PAH. In three of four cases mean blood pressure was slightly higher on standing but there was no great change. Since cardiac output is lower on standing than on lying, total peripheral resistance must be somewhat increased on standing. That the renal vascular bed participates in this constriction is shown by the fact

TABLE 1. Effect of high protein intake on clearances

SUBJECT DATE	URINE FLOW	PAH CLEARANCE	INULIN CLEARANCE	F.F.	PLASMA GLUCOSE	MEAN B.P.	RRU/M²
	cc/min/M²	cc/min/M²	cc/min/M²		mgm. %	mm. Hg	
	Uncontrolled diet (about 90 grams protein)						
HW 9/24/47	0.59	350	59	0.169		88	7.1
	0.82	395	62	0.157		84	6.0
	4 hours after large protein meal						
HW 11/4/47	6.22	380	67	0.176	73	88	6.6
	2.71	376	66	0.176	77	88	6.6
	1.81	373	51	0.137	77	87	6.6
	Protein intake 220 grams daily for week preceding 1/22/48						
HW 1/22/48	1.18	454	83	0.182	95	89	5.6
	1.08	427	72	0.168	75	89	5.9
	1.04	442	84	0.190	79	88	5.8
	Uncontrolled diet (about 90 grams protein)						
FC 10/15/47	1.15	335	58	0.172	88	89	7.5
	4.27	339	59	0.174	86	90	7.5
	4 hours after large protein meal						
FC 11/11/47	0.56	341	66	0.194	105	90	7.5
	0.63	333	67	0.20	94	91	7.7

that in every case renal vascular resistance is higher on standing than on lying. A single observation of a postural change in renal vascular resistance of these magnitudes would not be significant, but it is believed that the consistency of behavior justifies the view that the usual normal response on changing from the recumbent to the standing position is a slight increase in renal resistance, due to vasoconstrictor impulses rather than to adrenalin output.

Effects of cold. Three experiments were performed on three subjects; one of these has been omitted because great fluctuations in the control periods indicated that urine collections were not quantitative. All periods were in recumbency, the first two being controls, while cold was applied during the third period.

TABLE 2. POSTURAL EFFECTS ON CLEARANCES

SUBJECT DATE	POSTURE	URINE FLOW	PAH CLEARANCE	INULIN CLEARANCE	F.F.	PLASMA GLUCOSE	MEAN B.P.	RRU/M^2
		$cc/min/M^2$	$cc/min/M^2$	$cc/min/M^2$		*mgm. %*	*mm. Hg*	
HLW	lying	4.33	259	47.8	0.185			
5/1/47	lying	4.87	258	46.4	0.180			
	standing	3.66	228	46.5	0.204			
HW	lying	0.59	350	59.0	0.169		88	7.1
9/24/47	lying	0.82	395	61.8	0.157		84	6.0
	standing	0.73	283	48.8	0.173		92	9.2
RJP	lying	0.94	387	74.7	0.193		94	6.9
10/1/47	standing	2.13	304	61.5	0.202		96	9.0
	lying	0.78	318	59.1	0.186		94	8.4
FC	lying	1.15	335	58	0.172	88	89	7.5
10/15/47	lying	4.27	339	59	0.174	86	90	7.5
	standing	2.9	261	55	0.211	88	85	9.2
RO	lying	0.21	219	41	0.185		85	5.9
10/8/47	lying	0.24	319	52	0.162		86	4.1
	standing	0.25	245	57	0.231		92	5.7

With *HLW* the cold stimulus was applied by immersing a hand and about a third of the forearm in a mixture of ice and water at 0° C. The immersion was not continuous, since the procedure was too painful. Immersion was continued for a few minutes until the pain became intense; the hand was then withdrawn for a minute and the cycle repeated. In order to avoid the pain element, *subject FAH* dipped a hand and lower forearm into water at 14–15° C. To avoid the complication of adaptation, every three minutes the hand was removed and the other hand immersed, while the nonimmersed hand was dried and warmed by body contact; the cycle was repeated throughout the third clearance period. It is seen in table 3 that *HLW* (cold plus pain) showed a slight fall in both clearances, a questionable rise in filtration fraction, and a significant rise in mean blood pressure and renal vascular resistance. *Subject FAH* (cold alone) showed no change in any of the values. It is quite probable that exposure of a sufficiently large area of body surface to a temperature of 14–15° C. would result in significant renal vasoconstriction, but the threshold is obviously higher than that of the hand and forearm, since application of a very slight cold stimulus (evaporation of a few drops of ether) to one hand in a large series of student experiments has consistently produced striking vasoconstriction in the other hand.

Exercise. Eight experiments have been performed on six subjects; two of these are omitted because of great fluctuations, presumably due to inadequacy of urine collections, in the control periods. The results of the six satisfactory experiments are seen in table 4. All periods except the running were in recumbency. Blood pressures were taken at three- or four-minute intervals except during the period of running; they were not taken in the experiment on *HLW*. Blood pressure readings were not begun after exercise until the subject had emptied his bladder; the first postexercise reading was usually two or three minutes after exercise had stopped. On this reading the systolic pressure usually was higher and the diastolic lower than before exercise; the systolic then fell and the diastolic rose. Individual readings are not shown in the table; mean blood pressure indicates the average of the mean pressure values taken during a given period. Mean pressures during the periods after exercise have always been slightly lower than the pre-exercise control values; total peripheral resistance thus remains lowered for some time after exercise. Mean arterial pressure during exercise was not determined, but is known to be at least as high as at rest. The values for RRU/M² during exercise are given as minimum values, taking mean pressure during exercise as being the same as at rest.

TABLE 3. EFFECTS OF COLD ON RENAL CIRCULATION

SUBJECT DATE	PROCEDURE	URINE FLOW	PAH CLEARANCE	INULIN CLEARANCE	F.F.	MEAN B.P.	RRU/M²
		cc/min/M²	*cc/min/M²*	*cc/min/M²*		*mm. Hg*	
HLW	control	1.12	257	49.5	0.193	102	11.3
6/9/47	control	3.45	247	47.8	0.194	98	11.3
	cold	4.75	221	44.5	0.201	113	14.7
FAH	control	0.56	373	63	0.168	90	6.9
10/29/47	control	0.38	375	61	0.163	91	6.9
	cold	0.52	375	62	0.166	93	7.0

During light exercise (*HLW*) PAH and inulin clearances fell slightly and remained down for at least 40 minutes after exercise; the urine remained protein-free as tested by heat. During moderate to moderately severe exercise (*LHR*, 12/18/47 and *RCC*) both clearances fell; the return of PAH clearance toward normal may or may not be completed at the end of 50 to 60 minutes, the return of inulin clearance to normal being faster. Behavior of filtration fraction during and after exercise has not been consistent. During exercise of this degree renal

TABLE 4. Effects of exercise on renal circulation

SUBJECT DATE	PROCEDURE	URINE FLOW cc/min/M²	PAH CLEARANCE cc/min/M²	INULIN CLEARANCE cc/min/M²	F.F.	URINE ALBUMIN	PLASMA GLUCOSE mgm. %	MEAN B.P. mm. Hg	RRU/M²
	During 13 of the 15 minutes of second period subject jogged at 4.3 miles per hour. Respiration and pulse rate increased, slight sweating, not dyspneic or exhausted.								
HLW 3/11/47	lying	1.34	266	54	0.205	–			
	running	1.17	232	50	0.214	–			
	lying	1.34	222	47	0.213	–			
	lying	1.25	224	45	0.201	–			
	During 12 of the 14 minutes of third period subject ran at 6.2 miles per hour. Dyspneic, exhausted, sweating profusely.								
CML 11/12/47	lying	5.25	343	67	0.197	–	89	97	8.1
	lying	5.65	357	63	0.177	–	87	96	7.7
	running	1.60	74	17.6	0.237	–	112		37+
	lying	0.37	164	31.2	0.190	++		88	15.2
	lying	0.97	260	33.6	0.129	+	98	90	9.8
	lying	0.47	264	34	0.128	–	87	90	9.7
	During 11 of the 15 minutes of third period subject ran at 5.6 miles per hour. Slight dyspnea, not exhausted, moderate sweating.								
RCC 11/19/47	lying	0.52	375	59	0.157	–	98	97	7.4
	lying	0.53	429	56	0.131	–	97	94	6.2
	running	0.16	168	22	0.132	–	159		16+
	lying	0.87	267	46	0.174	+		86	9.1
	lying	0.36	326	57	0.174	–	122	87	7.5
	lying	0.98	396	57	0.145	–	104	85	6.1
	During 11 of the 13½ minutes of third period subject ran at 7.2 miles per hour. Dyspneic, exhausted, sweating profusely.								
LHR 11/18/47	lying	5.1	403	67	0.165	–	92	97	6.8
	lying	6.7	388	66	0.169	–	90	95	7.0
	running	1.73	79	13.6	0.174	–	190		35+
	lying	0.75	179	33	0.185	++		86	13.6
	lying	0.56	230	53	0.232	+	145	92	11.3
	lying	0.38	275	58	0.212	–	122	89	9.2
	During 19½ of the 20½ minutes of the third period subject ran at 5.7 miles per hour. Hyperpnea but not dyspnea, moderate sweating, not exhausted as on 11/18/47.								
LHR 12/18/47	lying	1.57	398	64	0.161	–	86	97	6.9
	lying	4.84	388	60	0.155	–	84	95	7.0
	running	1.67	178	38	0.214	–	100		15+
	lying	3.60	293	67	0.23	–		94	9.1
	lying	5.60	274	61	0.223	–	97	95	9.8
	lying	7.05	276	60	0.217	–	84	95	9.8
	During 20 of the 22 minutes of third period subject ran at 6.7 miles per hour. Dyspnea, sweating profusely, exhaustion almost as great as on 11/18/47.								
LHR 1/8/48	lying	5.8	340	58.6	0.17	–	83	96	8.0
	lying	6.31	357	61.5	0.17	–	83	93	7.4
	running	1.29	123	25.3	0.21	–	108	95+	22+
	lying	1.28	333	65.8	0.20	–		85	7.3
	lying	1.05	311	55.8	0.18	–	94	85	7.8
	lying	2.87	328	54.1	0.17	–	85	87	7.5

given subject the response is a vasoconstriction in all the channels, with no ischemic and functionless glomeruli and therefore no postexercise proteinuria, while above this critical level glomeruli begin to drop out of function, in increasing numbers as the work level is increased, so that when glomerular function is re-established after exercise protein appears in the filtrate of these reversibly damaged glomeruli. Significant dropping out of glomeruli does not begin until renal blood flow has first been reduced to below half of the resting value. Since in normal subjects the critical level of work required to cause any significant number of glomeruli to drop out is higher than can be maintained for more than a few minutes, the kidneys are automatically protected against prolonged ischemia during prolonged periods of work.

The border line between the work level for a given subject which is followed by proteinuria and that which is not is quite sharp. Thus, with *LHR*, running at 7.2 miles per hour for 11 minutes brought out protein in the first two postexercise samples, while 6.7 or 5.7 miles per hour for 20 minutes failed to produce proteinuria. For *CML* (table 4 and figure 2) the effect of running 6.2 miles per hour for 12 minutes was as great as was 7.2 miles per hour for 11 minutes with *LHR*, and *CML* showed protein in the first two postexercise samples. Running at 5.6 miles per hour was somewhat less strenuous for *RCC*, who showed protein only in the first postexercise sample. *Subject LHR* was in the best physical condition, was capable of greater work performance, and required a higher level of performance to bring on proteinuria. The speed of running for a period of a few minutes necessary to bring on proteinuria would probably show good correlation with other criteria of physical fitness, the threshold speed for the less fit being about 5.5 or less and for the most fit 7 or more miles per hour.

These findings thus support, although they do not prove, the view that under great stress many glomeruli may cease functioning, a view recently restated with additional evidence (10). They indicate that such dropping out of glomerular function occurs only under great stress and that the usual degrees of exercise cause a generalized renal vasoconstriction without determinable dropping out of glomeruli. Evidence against glomerular intermittence as a normal occurrence in the anesthetized dog and rabbit has been obtained on the basis of completeness of renal vascular injection with India ink (11). Constancy of glucose Tm indicates absence of glomerular intermittence in man at rest, during diuresis or periods of renal hyperemia, or under the influence of various drugs, although a sufficiently large dose of adrenalin can close many glomeruli (12).

DISCUSSION

It is shown that the renal vascular bed constricts in response to the normal stimuli of exercise and that the degree of constriction is proportional to the severity of exercise, the resistance being increased at least five-fold during heavy exercise. Cutting down of the renal blood flow from a liter or more per minute at rest to 200–250 cc. per minute during exercise contributes about a liter of extra blood flow to the active regions. In so far as a rise in plasma glucose may be taken as an index of adrenalin output, it appears that significant increase in adrenalin output does not occur until exercise reaches a high level. The renal vasoconstriction seen on light to moderate exercise is apparently due to vasoconstrictor nerve impulses, the constricting action of adrenalin on the renal vessels being added as exercise beomes more severe. The renal vasoconstriction of maximum exercise for relatively brief periods may progress to the point where many glomeruli drop out of function, as indicated by postexercise proteinuria; whether this comes on only when adrenalin output is increased cannot be stated with certainty.

A further possibility, suggested by Trueta's work (10), that during exercise the PAH plasma clearance is no longer a measure of renal plasma flow because of shunting of blood through regions which do not extract PAH, may be considered. According to Trueta, stimulation of the splanchnic nerve or of the renal nerve plexus, or massive intravenous doses of adrenalin may, particularly in the rabbit, cause selective constriction of the cortical vessels to complete or almost complete closure, with diversion of blood through the juxtamedullary glomeruli and the vasa recta springing from their efferent arterioles. Our findings show conclusively that the 'effective' renal plasma flow is reduced but do not tell whether the constriction is confined to the cortical vessels. Nor do they tell whether 'effective' renal plasma flow becomes less than total renal plasma flow, due to diversion of blood into noneffective renal tissue. If Trueta's concept of the diversion channels is correct, some of the diverted blood passes "from the 'arcuate' and proximal parts of the interlobular arteries, via the juxtamedullary glomeruli, to the corresponding veins without traversing a capillary *network*" (p. 64). This would result in less complete PAH extraction and PAH plasma clearance, while still a measure of 'effective' renal plasma flow, would no longer be a measure of total renal plasma flow. Our experiments do not exclude the possibility of diversion through noneffective channels, but since the fall in PAH clearance increases progressively with increasing exercise levels, the results seem more simply explained on the basis of progressive vasoconstriction, without invoking the opening of shunts. A positive answer could be obtained by observing, through renal vein catheterization, whether the renal extraction of PAH is diminished on exercise; such experiments have not been attempted.

SUMMARY

1. Increase in protein intake at a single meal had no significant influence on PAH clearance or on renal vascular resistance in normal man and slightly raised inulin clearance; 220 grams of protein daily for a week raised PAH clearance 18 per cent and inulin clearance 31 per cent, and lowered renal vascular resistance 12 per cent.

2. PAH clearance averaged 19 per cent lower and inulin clearance 8 per cent lower on standing than in recumbency; renal vascular resistance averaged 22 per cent higher on standing, due to vasoconstrictor impulses rather than to increased adrenalin output.

3. Immersion of a hand in painfully cold ice water at 0° C. produced a slight fall in PAH and inulin clearances with a slight rise in mean blood pressure; a pure

cold stimulus through hand immersion in water at 14–15° C. had no effect on clearances or mean blood pressure in the one normal subject studied.

4. Light exercise produces slight falls in PAH and inulin clearances. Moderate to moderately severe exercise lowered PAH and inulin clearances to half or less of their resting values and at least doubled renal vascular resistance; protein may appear in the first postexercise urine samples. Brief maximum exercise lowers PAH and inulin clearances to 20 per cent or less of their resting values while renal vascular resistance is increased at least five-fold; a longer period of maximum exercise at a lower rate is less effective, reducing clearances to about a third of their resting values. Rise in plasma glucose, taken as a tentative index of increased adrenalin output, usually is not marked unless exercise is severe; in a given subject it is proportional to the severity of exercise.

5. Protein did not appear in the urine formed during even the severest exercise of these experiments but after severe exercise was present in the first one or two postexercise samples. The interpretation of the findings is that exercise brings on generalized renal vasoconstriction and that only at the maximum work levels do any large number of glomeruli drop out of function, occurrence of postexercise proteinuria being taken as an index of such dropping out during exercise.

REFERENCES

1. Smith, H. W. Harvey Lectures **35**: 166, 1939.
2. Smith, H. W. University of Kansas Lectures. 1943, p. 58.
3. McCann, W. S. and M. J. Romansky. Jour. Am. Med. Assoc. **115**: 573, 1940.
4. Brun, C., E. O. Knudsen and F. Raaschou. Acta Med. Scand. **122**: 315 and 332, 1945.
5. Brun, C., E. O. Knudsen and F. Raaschou. J. Clin. Invest. **25**: 568, 1946.
6. Wolf, G. A. Trans. Assn. Nerv. Ment. Dis. **23**: 358, 1943.
7. Barclay, J. A., W. T. Cooke, R. A. Kenney and M. E. Nutt. J. Physiol. **104**: 14P, 1945. This Journal **148**: 327, 1947.
8. Findley, T. and H. L. White. Proc. Soc. Exp. Biol. Med. **45**: 623, 1940.
9. White, H. L., I. T. Rosen, S. S. Fischer and G. H. Wood. This Journal **78**: 185, 1926.
10. Trueta, J., A. E. Barclay, P. M. Daniel, K. J. Franklin and M. M. L. Prichard. Studies of the Renal Circulation. Springfield, Ill., Charles C Thomas.
11. White, H. L. This Journal **128**: 159, 1939.
12. Smith, H. W. University of Kansas Lectures, 1943, p. 93.

DURATION OF RENAL ISCHEMIA REQUIRED TO PRODUCE UREMIA[1]

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Bywaters and his colleagues (1) early in the late war drew attention to the fact that shock from crush injuries and other traumata is not infrequently followed by renal failure that persists after other signs of shock have disappeared and can result in fatal uremia. Experiments from this laboratory, presented in a preliminary report in 1944 (2), and later detailed (3), showed that severe hemorrhagic or traumatic shock in dogs could cause almost complete cessation of renal blood flow. Lauson, Bradley and Cournand (4) at about the same time reported decreased renal blood flow in human cases of shock. Since these effects could occur while the arterial blood pressure was as high as 80 to 100 mm. it appeared that the decrease in renal blood flow was due to vascular constriction (2, 3). Of the different hypotheses that had been advanced concerning the cause of the renal damage produced by shock (see Lucké, 5), the demonstration of decreased renal blood flow supported the hypothesis that renal ischemia existing during the shock state is a cause of subsequently persisting renal failure (2, 3).

To test this hypothesis further, renal ischemia was produced in dogs (2) by ligating the renal arteries for varying periods, and the after effects on renal function were observed. Ischemia of two-hours' duration was followed by temporary depression of urea clearance to nearly zero, but gradual recovery followed during a period of two or three weeks, and renal function might be completely restored. Ischemia of over four-hours' duration uniformly caused irreversible renal injury leading to death in uremia in four to eight days. Histological examinations by Dr. Jean Oliver showed that the glomeruli appeared to be normal, but that, in the distal tubules, there were changes resembling those seen in cases of uremia following the crush syndrome.

The present paper presents details of, and additions to, the original experiments performed to determine the duration of renal ischemia necessary to cause irreversible damage. As will be seen, dogs survived renal ischemia for two hours.

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Depending on environmental temperature, nutritional state of the animal, etc., three hours of renal ischemia might or might not be fatal.

At the time these experiments were undertaken, except for one paper by McEnery, Meyer and Ivy (6), no data could be found in the literature to indicate the duration of renal ischemia required to cause irreversible injury of the kidneys in dogs. McEnery *et al.* state, "Dogs may or may not survive a 30- to 60-minute period of ischemia of both kidneys". Scarff and Keele (7), confirmed recently by Badenoch and Darmady (8) found that one or two hours of occlusion of the renal artery produced irreversible renal damage in rabbits. To judge by comparison with our results, irreversible damage to the kidneys is caused by ischemia of somewhat shorter duration in rabbits than in dogs.

METHODS

1. *Method of producing renal ischemia.* The following procedure was applied with aseptic precautions to a series of female dogs under sodium pentobarbital anesthesia.

Through a midline abdominal incision the right kidney was excised. The left kidney was mobilized by dividing between silk ligatures the parietal peritoneum and the accompanying capsular vessels about one inch from the kidney. The renal artery was dissected free down to its origin from the aorta for a distance of 1½ to 2 cm. Two serrefines, with jaws covered by rubber tubing and with springs of sufficient strength to obliterate the arterial lumen without damaging the intima, were then applied to the renal artery. The time of application of the serrefines was noted and the wound closed in layers with silk. In none of our experiments was the renal vein or the ureter obstructed. The presence of the ureteral vein and artery was noted at operation but it was felt unwise to ligate the ureteral artery even though in some instances it was obvious that some blood was still entering the kidney through this small vessel.

Following the removal of the serrefines the kidney was tacked in its original position by mattress sutures placed through the renal peritoneum and the dorsal abdominal wall at the four poles of the kidney. The abdominal incision was closed with layered silk sutures.

2. *Chemical determinations.* The blood urea nitrogen concentration was followed in all experiments by the manometric hypobromite method of Van Slyke and Kugel (9). In experiments performed in the months of June and July 1944 continuous 24-hour urea clearances were also performed. Urine urea determinations were made by the method of Van Slyke and Cullen (10). In this series of experiments urea clearances were performed before the serrefines were applied to the renal artery and for the two hours after release of the serrefines, at which time the animal was still under sodium pentobarbital anesthesia. Subsequently the animals were placed in metabolic cages and 24-hour collections of urine obtained. The urea clearances were followed until the death of the animal or until the clearance had returned to the normal range.

RESULTS

Experiments Performed in January, February and April 1943

1. *Renal ischemia for one hour.* Six dogs subjected to renal ischemia for one hour survived more than 30 days.

2. *Renal ischemia for two and three hours.* Six dogs were subjected to renal ischemia for two hours and six dogs were subjected to renal ischemia for three hours. One animal in this series died on the fourth post-operative day with a large *B Coli* abdominal abscess, the remainder of the dogs in this series survived more than 30 days.

3. *Renal ischemia for four hours.* Of six dogs subjected to renal ischemia for four hours, three lived for more than 30 days; three dogs died after 4, 4 and 10 days with blood urea nitrogen concentrations at death of 157, 182 and 320 mgm. per 100 cc., respectively.

4. *Renal ischemia for six hours.* Two dogs were subjected to renal ischemia for six hours. Both died in uremia after 4 and 10 days, respectively.

5. *Bilateral nephrectomy.* Two dogs were subjected to removal of both kidneys. They died on the fourth and eighth day, respectively, in uremia, verifying the observations of Hoff, Smith and Winkler (11).

Experiments Performed in June and July 1944

1. *Renal ischemia for two hours.* Five dogs were subjected to renal ischemia for two hours; all survived for over 30 days.

2. *Renal ischemia for three hours.* Of five dogs subjected to renal ischemia for three hours, two dogs survived for over 30 days; the three dogs that died survived 6, 9 and 15 days and the blood urea nitrogen concentrations at death were 374, 361 and 462 mgm. per 100 cc., respectively.

In our first series of experiments with renal ischemia of three hours' duration it will be noted that the experiments were performed in the winter and spring of the year when the environmental temperature was never above 80° F., while the second series of experiments were performed in the summer months when the environmental temperature was often above 95° F. and the humidity was also greatly increased. It appears that the increased environmental temperature and humidity in the summer months may have been responsible for the increased mortality of the dogs subjected to three hours of renal ischemia during this time of the year (cf. Lusk, 12, p. 151–155).

Blood Urea Changes

In figure 1 the plasma urea nitrogen concentration changes in one animal which had a bilateral nephrectomy are compared with the plasma urea nitrogen changes in three dogs subjected to renal ischemia of three hours' duration. The nephrectomized dog, *K-7*, died on the fifth day. *K-91* and *K-90*, subjected to three hours of renal ischemia, died on the sixth and fourteenth day, respectively. These experiments were performed in the summer months. *K-11*, subjected to three hours of renal ischemia showed no tendency for improvement of the urea clearance, after the fifth day there was a slow downward trend.

SUMMARY

1. Dogs with the right kidney removed uniformly survived clamping of the left renal artery for two hours. Some dogs survived clamping of the renal artery for three or four hours. Death in uremia regularly ensued with longer clamping of the renal artery.

2. In dogs subjected to three hours of renal ischemia the mortality was greater during summer, with high environmental temperature and humidity, than during winter, with low temperature and humidity.

3. After the clamp was removed from the renal artery, the urea clearance was extremely low, of the order of one to 10 per cent of normal, and blood urea began to rise rapidly. If recovery ensued, a steady rise of renal function occurred as measured by the urea clearance, and the clearance eventually reached a normal level, although a month was sometimes required for it to do so. If recovery did not take place, renal function remained at a low level, with progressive rise in blood urea nitrogen terminating in uremia and death.

REFERENCES

1. Bywaters, E. G. L. and D. Beall. Brit. Med. J. **1:** 427, 1941; Beall, D., E. G. L. Bywaters, R. H. R. Belsey and J. A. R. Miles. Brit. Med. J. **1:** 432, 1941; Bywaters, E. G. L. Brit. Med. J. **1:** 643, 1942.
2. Van Slyke, D. D., R. A. Phillips, P. B. Hamilton, R. M. Archibald, V. P. Dole and K. Emerson, Jr. Trans. Assn. Am. Phys. **58:** 119, 1944.
3. Phillips, R. A., V. P. Dole, P. B. Hamilton, K. Emerson, Jr., R. M. Archibald and D. D. Van Slyke. This Journal **145:** 314, 1946.
4. Lauson, H. D., S. E. Bradley and A. Cournand. J. Clin. Inv. **23:** 381, 1944.
5. Lucke, B. The Military Surgeon, **99:** 371, 1946.
6. McEnery, E. T., J. Meyer and A. C. Ivy. J. Lab. Clin. Med. **12:** 349, 1927.
7. Scarff, R. W. and Keele, L. A. Brit. J. Exp. Path. **24:** 147, 1943.
8. Badenoch, W. W. and Darmady, E. M. J. Path. and Bact. **59:** 79, 1947.
9. Van Slyke, D. D. and V. H. Kugel. J. Biol. Chem. **102:** 489, 1933.
10. Van Slyke, D. D. and G. E. Cullen. J. Biol. Chem. **24:** 117, 1916.
11. Hoff, H. E., P. K. Smith and A. W. Winkler. J. Clin. Inv. **20:** 607, 1941.
12. Lusk, G. The Science of Nutrition. 4th ed. Philadelphia, W. B. Saunders, Co., 1928.
13. Rhoads, C. P., A. S. Alving, A. Hiller and D. D. Van Slyke. This Journal **109:** 329, 1934.

EFFECT OF 20, 60 AND 120 MINUTES OF RENAL ISCHEMIA ON GLOMERULAR AND TUBULAR FUNCTION[1]

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In other papers (1, 2) it has been demonstrated that renal ischemia of two-hour duration, caused by clamping the renal arteries of dogs, although not quite sufficient to cause irreversible injury, was followed by a period during which the urea clearance was depressed to a small fraction of its normal value, the blood urea nitrogen rose, and normal function was regained only in the course of two or three weeks.

The fall in urea clearance might be due to 1) decrease in the renal blood flow, or 2) decrease in the proportion of plasma water, with its urea and other crystalloid solutes, filtered in the glomeruli or 3) increase in reabsorption of urea in the tubules, such as might occur if the tubular walls were so devitalized by the ischemia that they could not prevent back diffusion of urea with reabsorbed water.

In an attempt to determine which of the three factors were responsible, experiments of the following type were performed. The right kidneys of female dogs were removed, and the dogs were infused with a solution of para-amino hippuric acid (hereafter designated as PAH) and creatinine, as described in a previous paper (3). The left kidneys were subjected to ischemia by clamping the renal arteries for periods of 20, 60 or 120 minutes. At intervals up to two hours after removal of the clamps blood was drawn simultaneously from the renal artery and the renal vein, analyses of the plasma from these bloods were made for creatinine and PAH and the rates of excretion of both substances in the urine were determined.

Of the three factors considered as possible causes for the persisting low urea clearance after renal ischemia, the results of the experiments could be expected to give indications as follows.

1. Renal blood flow could be calculated from the PAH values by the Fick principle, as (PAH excreted per minute)/(PAH removed by the kidneys from one cc. of renal blood). This principle of measuring renal blood flow was introduced by Van Slyke, Rhoads, Hiller and Alving (4), who used urea as the measured

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research. The results were in part presented in 1944 by the authors and collaborators (1) in a report on the effects of shock on the kidney. The Bureau of Medicine and Surgery of the U. S. Navy does not necessarily undertake to endorse views or opinions which are expressed in this paper.

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excretory substance. However, since only about 12 per cent of the blood urea is extracted by the kidneys, while an average of 87 per cent of plasma PAH is extracted (1, 3, 5) the PAH extraction can be measured more accurately. Previous experiments (1, 3) have shown that consistent values for renal plasma flow are obtained by the Fick principle, with PAH as excretory substance.

2. The fraction of plasma water filtered could be estimated from the fraction of creatinine extracted from the renal plasma, *provided* that the ischemia does not so injure the tubules that they permit some of the creatinine filtered in the glomerulus to diffuse back into the blood with the water reabsorbed from the tubular umina. Previous studies (6) have indicated that creatinine in the normal dog is excreted entirely by glomerular filtration, without significant tubular reabsorption.

3. The functional state of the tubular cells could be indicated by the completeness with which the kidneys remove PAH from the renal blood plasma. Since normally an average of 87 per cent is removed (1, 3) while only 20 to 30 per cent can be considered to be filtered in the glomerulus (6), it follows that the greater part of the PAH removed from the plasma is normally excreted by the tubules. If, after renal ischemia, the tubules can at once resume extraction of PAH so complete that 87 per cent is removed from the renal plasma, it can be assumed as probable that the tubular cells are undamaged. If, however, there is a severe decrease in the PAH extraction, it may be taken as evidence of deranged tubular function and would suggest the possibility that the renal malfunction may include increased permeability to back diffusion of urea from the tubular lumina to the blood. Normally the tubules limit back diffusion of urea to about 40 per cent of that filtered (6, 7).

METHODS

1. *Operative and experimental procedures.* Female dogs were used. After anesthetizing with sodium pentobarbital, the spleen and right kidney were excised through a midline abdominal incision. The peritoneum and adventitia overlying the left renal vein were then dissected free from the vein so that samples of blood could be readily obtained. The parietal reflection of renal peritoneum was then excised between the upper and lower poles of the left kidney to permit exposure and dissection of the artery for a distance of one half to two cm., sufficient to accommodate the serrefines applied subsequently for the production of renal ischemia. A urethral catheter was inserted into the bladder and strapped in place. A ureteral catheter was inserted into the fore-limb vein and was connected to a continuous intravenous infusion pump, for the infusion of the solution of creatinine and PAH (3).

The technique for obtaining blood and urine samples was as described by Phillips *et al.* (3). Blood was drawn from the renal vein with a syringe provided with a curved No. 20 hypodermic needle; the needle was bent in a 90° curve so that its point was at right angles to the syringe axis. This facilitated puncturing the renal vein to obtain the sample. Care was taken to avoid undue tension on the kidney, its vessels and the ureter at the time of sampling, and in addition care was taken to avoid any tension on the abdominal vena cava at the time of sampling.

In these experiments it was of interest to follow the clearance and extraction of creatinine and PAH immediately after release of the serrefines applied to the renal artery. Consequently the peritoneum and capsular vessels at the upper and lower pole of the kidney were left undivided, to insure that the kidney would remain in its original position and that kinking of the ureter, renal vein and renal artery would be minimized. As pointed out in the preceding paper (2), some blood does enter the kidney by way of the capsular vessels (not divided in these experiments) and in some animals by way of the ureteral artery. However, the amount of such vascular supply is small compared to the supply obtained through the renal artery.

Priming, followed by sustaining, infusions of creatinine and PAH were given, and one hour was allowed for attainment of equilibrium of creatinine and PAH between plasma and cells (3). One hour after start of the sustaining infusion three urine samples, collected during successive periods of 10 to 15 minutes each, were obtained. Blood was drawn from the femoral artery before the start of the first urine period and at the end of the third urine period. A sample of renal venous blood was obtained in the middle of the second urine period. After these control observations serrefines with rubber-covered jaws were applied to the renal artery for varying periods of time, which, in 3 series of experiments were approximately 20 minutes, 60 minutes and 120 minutes respectively. Ten minutes after release of the serrefine a sample of arterial blood was drawn and urine samples were obtained every 15 minutes for the subsequent two or more hours. Arterial blood samples were obtained at frequent intervals from the femoral artery. Renal venous blood samples were obtained at approximately 20, 60 and 120 minutes after release of the serrefines.

During application of the serrefines the sustaining infusion was discontinued. When renal ischemia was maintained for 120 minutes bladder washings for 110 minutes of this time were obtained. The smallness of the amounts of PAH found in these bladder washings, obtained during the period of ischemia, was a further check on the completeness of the renal ischemia. In two experiments both kidneys of each dog were excised and the bladder clearance of PAH (clearance estimated from PAH in bladder washings of the nephrectomized dogs) was determined with plasma PAH concentrations of 4–6 mgm. per 100 cc. The bladder clearance was found to be in the range of 0.02–0.04 cc. per minute. In the experiments here reported the PAH clearance during 120 minutes of renal ischemia were never more than 10 times the bladder clearance, indicating that the renal ischemia was nearly complete.

2. *Method of administration and analysis of creatinine and PAH.* The techniques used for administering creatinine and PAH and for analyzing plasma, blood and urine for these substances have been described previously (3). PAH concentrations found in plasma of renal venous blood were corrected as previously described (3), for diffusion of 5 per cent of the plasma PAH *in vitro* from plasma to erythrocytes during the drawing and centrifuging of the blood.

RESULTS AND DISCUSSION

Renal blood flow. The results in table 1 indicate that, after renal ischemia for periods up to two hours, when the clamps were removed from the renal arteries blood flow through the kidneys was soon reestablished. Renal plasma flows, measured 90 to 120 minutes after release of the clamps, averaged 81 to 85 per cent of the pre-ischemic flows, whether the period of ischemia had been 20, 60 or 120 minutes. The decrease of urea clearance nearly to zero, noted previously (1) as a sequence of clamping the renal artery for two hours, was evidently not due to failure of blood flow to return to the kidneys after the clamps were removed.

TABLE 1. *Effects of clamping renal artery for varying lengths of time on subsequent renal function (one-kidney dogs)*

DURATION OF CLAMPING	DOG NO.	% OF EXCRETORY SUBSTANCE EXTRACTED FROM RENAL PLASMA						RENAL PLASMA FLOW[1]			RENAL PLASMA FLOW ESTIMATED AS 1.15 x PAH CLEARANCE		
		Para-amino hippurate			Creatinine								
		Before clamping renal artery	15–20 min. after release of clamp	90–120 min. after release of clam p	Before clamping	15–20 min. after release of clamp	90–120 min. after release of clamp	Before clamping	15–20 min. after release of clamp	90–120 min. after release of clamp	Before clamping	15–20 min. after release of clamp	90–120 min. after release of clamp
min.								*cc/min.*	*cc/min.*	*cc/min.*	*cc/min.*	*cc/min.*	*cc/min.*
20	N2	0.91	0.85	0.88	—	—	—	157	96	48	164	94	49
20	N3	0.85	0.79	0.73	0.33	0.21	0.21	48	85	85	47	97	71
20	N4	0.80	0.57	0.61	0.30	0.23	0.38	33	22	18	30	14	13
20	N9	0.90	0.90	0.72	0.17	0.22	0.26	61	58	46	63	60	38
Mean % of value before clamping		100	90	85	100	90	118	100	101	85	100	91	71
60	N6	0.76	0.39	0.50	0.33	0.15	0.20	67	37	66	59	17	38
60	N7	0.83	0.67	0.71	0.22	0.16	0.25	99	98	76	94	29	46
60	N8	0.81	0.31	0.38	0.22	0.05	0.15	96	67	72	90	24	31
Mean % of value before clamping		100	57	66	100	47	72	100	75	84	100	29	49
120	K69	0.90	0.11	0.11	0.23	0.02	0.02	87	57	76	90	7	9
120	K67	0.91	—	0.20	0.19	—	0.05	93	—	56	96	—	13
120	K68	0.94	—	0.43	0.22	—	0.14	42	—	36	46	—	17
120	K70	0.92	0.16	0.13	—	—	—	116	52	90	122	9	14
Mean % of value before clamping		100	—	24	100	—	37	100	—	81	100	—	18

[1] Renal plasma flow measured as (mgm. PAH excreted per min.) / (mgm. PAH extracted from 1 cc. of plasma).

PAH extraction and tubular damage. The PAH extraction values indicate that tubular function was severely deranged by two hours of ischemia. The damage was progressive with the duration of the ischemia, as indicated by the progressive decrease in the fraction of PAH extracted from the renal plasma; this fraction averaged 85 per cent of its pre-ischemic value after 20 minutes ischemia, 66 per cent after 60 minutes and 24 per cent after 120 minutes of ischemia, the extractions being averages measured 90–120 minutes after release of the arterial clamps. This functional evidence of tubular damage agrees with the results of histological examination of the kidneys by Dr. Jean Oliver, mentioned in a previous report (1).

Tubular reabsorption as a cause of renal failure after renal ischemia and shock. The functional effects of tubular injury have been studied by Richards (8), who observed *in vivo* the activities of the nephrons of frogs that had been poisoned by mercury and other nephrotoxic substances. He found that in the glomeruli blood flow and filtration went on at a fully normal rate, but that the filtrate was completely absorbed from the tubular lumina, so that no urine reached the bladder. Anuria resulted, not from failure of filtration, but from complete reabsorption of the filtrate. The poisoned tubular walls permitted unselective diffusion of the entire filtrate, presumably drawn by the osmotic attraction of the plasma proteins, back into the circulation. The tubular walls apparently had become devitalized, so that they acted like inert permeable membranes.

In view of the absence of evidence of marked glomerular or vascular injury and of the positive evidence, functional and histological, of tubular injury, it appears probable that tubular reabsorption of the type observed by Richards (8) causes the urea retention that follows sufficiently prolonged periods of renal ischemia, as exemplified by figure 1 (reversible) and figure 2 (irreversible) of the preceding paper (2).

Lucké (9) has noted in kidneys from cases of uremia following shock from war injuries that the type of tubular injury was identical with that observed in cases injured by nephrotoxic poisons, and points out, from analogy with Richards' observations, that post-shock uremia is probably the effect of tubular injury caused by ischemia suffered during the shock, the renal failure being due to unselective tubular reabsorption of the type observed by Richards. Badenoch and Darmady (10) have compared side by side sections from the kidneys of patients dying in post-shock uremia with sections from the kidneys of rabbits in which uremia was caused by two-hour ligation of the renal arteries, and have found the type of tubular lesions practically identical. Results in previous papers from this laboratory (3) have shown that hemorrhagic and traumatic shock of severe grade causes nearly complete cessation of blood flow through the kidneys of dogs.

These observations, taken with the present data showing that ischemia prolonged over periods of hours comparable to the duration of shock in severe untreated cases produces progressive damage to tubular function, indicate the probability that post-shock uremia is caused by reabsorption of excretory products through damaged tubular walls. It also appears, from Lucké's (9) observation of the identical nature of the tubular lesions in post-shock uremia and in poisoning by sulfonamides and other nephrotoxic agents, that ischemia is one of many nephrotoxic agents that produce a common picture of tubular damage with resultant renal failure from tubular reabsorption.

Cause of the decreased extraction of creatinine from the renal plasma after ischemia. In the kidneys of the normal dog excreting ordinarily large volumes of urine there is evidence that creatinine and inulin do not pass to a measurable extent in either direction through the tubular walls, so that the fraction of either removed from the renal plasma is the fraction that is filtered out in the glomeruli (6, 11). It has been rather generally assumed that these relations hold also in abnormal kidneys, the clearance of creatinine or inulin in dogs and inulin in man serving as a measure of glomerular filtration. On such an assumption, a great decrease in the extracted fraction of creatinine, such as is seen in table 1 after two-hour ischemia, without a commensurate decrease in blood pressure, would be taken as evidence of decreased filtration due to decreased blood pressure in the glomerular capillaries, the fall in pressure being attributable to dilatation of the efferent vessels from the glomeruli.

However, in view of the evidence of tubular damage in these animals, of Richards' (8) evidence that such damage can cause abnormal tubular reabsorption, and in view of the lack of evidence of efferent dilatation, it appears more probable that the decrease in the filtered fraction of creatinine is attributable to back diffusion of creatinine through the injured tubular walls into the circulation. The condition appears to be similar to that found by Richards, Westfall, and Bott (12) in a dog with kidneys previously damaged by uranium. Creatinine clearances were consistently lower than inulin clearances. The results were interpreted by Richards *et al.* as evidence of back diffusion of creatinine through the injured tubular walls, inulin back diffusion being less because of lesser diffusibility of the large inulin molecule.

Interpretation of clearances as measures of renal blood flow and glomerular filtrate in conditions of tubular injury. Renal blood flow. Smith and his colleagues have shown for normal men and dogs that the clearances of diodrast (13) and PAH (14) approach so nearly to otherwise estimated values of the renal plasma flow that it is evident that their extractions from the renal plasma must be nearly complete, and that their clearances, in terms of cc. of plasma cleared of diodrast or PAH per minute, may therefore be assumed to approximate the volume of plasma flowing through the kidneys. These conclusions have been confirmed for subjects with normal kidneys; in both dogs (1, 3) and men (5) simultaneous determinations of PAH in arterial and renal venous plasma have shown that an average of 87–88 per cent of the PAH is normally extracted by the kidneys. Hence, when the kidneys are normal, one can estimate the renal plasma flow as 1.15 times the plasma PAH clearance, usually with but a small per cent error (3). This relation was shown to hold in acute hemorrhagic and traumatic shock so severe that the renal blood flow was temporarily reduced to a small fraction of normal (3). However, the results in the present paper show that when renal ischemia is sufficiently complete and prolonged to cause severe tubular injury the relation no longer holds. Comparison of the last three columns of table 1 with the preceding three columns shows that 1.15 times the PAH clearance approximately equaled the measured renal plasma flow in observations made before the renal artery was clamped, and after release of the clamps when the latter had been applied for only 20 minutes. But when the clamps had been applied for two hours the subsequent values of 1.15 times PAH clearance averaged less than one fourth of the measured renal plasma flow. It is evident that when the tubules are injured PAH extraction may be so diminished that the PAH clearance indicates only a small fraction of the renal blood flow. Presumably the same limitation applies also to diodrast clearances. Smith has been careful to state that PAH and diodrast plasma clearances will fall short of renal plasma flows in proportion as the fraction of PAH or diodrast extracted from the renal plasma falls short of unity. The present results indicate the need of keeping this reservation in mind, particularly in conditions where tubular injury may be present.

Glomerular filtrate. In normal dogs there is evidence that the tubular walls neither excrete nor reabsorb creatinine and inulin (6); hence the fraction of either extracted from the renal plasma by the kidneys may be taken to be the fraction filtered in the glomeruli. On the evidence of the classical experiments of Richards and his collaborators (15) showing that plasma crystalloids and water are filtered in the same proportions that they have in the plasma, the fraction of creatinine or inulin extracted can be taken as a measure also of the fraction of plasma water filtered, as long as the tubular walls retain their normal impermeability to the creatinine and inulin. When the tubules are damaged, however, so that they cannot extract PAH with normal completeness from the plasma, it appears possible that the tubular walls may become sufficiently permeable to creatinine and inulin to permit back diffusion of these substances from the tubular lumina into the circulation, in the manner observed by Richards (8), so that the amounts excreted are much less than those filtered in the glomeruli. For reasons discussed above, we are inclined to interpret the very low extractions of creatinine noted after two hours ischemia (table 1) to tubular back diffusion. Although the lack of an independent measure of glomerular filtration makes the conclusion less certain than those reached with regard to the interpretation of PAH clearances as renal blood flows in the same experiments, it appears that interpretation of clearances of creatinine, and of such similarly excreted substances as inulin, mannitol, and thiosulfate, in terms of glomerular filtration rates is open to doubt in conditions where tubular damage may make reabsorption of these substances a possibility.[3]

It is of interest in this connection that the impermeability of even normal tubular walls to creatinine, in the case of man, appears to be not absolute. Chesley (16) has found that when dehydration shrunk the urine flow of human subjects below 0.35 cc. per minute, reabsorption of all the urine solutes, including creatinine, occurred.

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[3] In a publication from this laboratory (3) on the effects of acute shock on renal function of dogs, the fraction of creatinine extracted from the renal plasma was used as a measure of the filtered fraction of plasma water. In this case the assumption was presumably justified, since simultaneous determinations of the extracted fraction of PAH showed that this fraction was normal, 0.87 ± 0.04, indicating that the degree of shock used had not yet significantly injured the tubules.

SUMMARY

Dogs have been subjected to renal ischemia by clamping the renal arteries for periods varying from 20 minutes to two hours, and the effects on the renal function have been studied by observations of the renal plasma flow and of the completeness with which para-amino hippurate and creatinine were extracted from the renal plasma during two hours after removal of the clamps.

Renal blood flow was quickly resumed at a nearly pre-ischemic rate.

The proportions of para-amino hippurate and creatinine extracted from the plasma were not markedly affected after 20-minute ischemia, but after two-hour ischemia they were reduced, the creatinine extraction, in 3 experiments, to 63, 26, and 9 per cent respectively of pre-ischemic values, the PAH extraction, in 4 experiments, to 37, 14, 11, and 10 per cent, respectively.

From the functional and histological effects of the two-hour ischemia, it appears probable that the decreases in extracted fractions were due to tubular injury, which decreased the proportion of plasma PAH excreted by the tubules, and increased tubular reabsorption of creatinine from the glomerular filtrate. The evidence supports Lucké's view that post-shock uremia is the result of tubular reabsorption of excretory products.

With tubular injury such as that caused by ischemia, PAH clearance does not serve as a measure of renal blood flow; and it appears doubtful that in the presence of such injury clearances of creatinine and similarly diffusible substances can be interpreted as measures of glomerular filtration rate.

REFERENCES

1. Van Slyke, D. D., R. A. Phillips, P. B. Hamilton, R. M. Archibald, V. P. Dole and K. Emerson, Jr. Trans. Assn. Am. Phys. **58:** 119, 1944.
2. Hamilton, P. B., R. A. Phillips and A. Hiller. This Journal **152:** 517, 1948.
3. Phillips, R. A., V. P. Dole, P. B. Hamilton, K. Emerson, Jr., R. M. Archibald and D. D. Van Slyke. This Journal **145:** 314, 1946.
4. Van Slyke, D. D., C. P. Rhoads, A. Hiller and A. Alving. This Journal **109:** 336, 1934.
5. Warren, J. V. and E. S. Brannon. Science **100:** 108, 1944.
6. Van Slyke, D. D., A. Hiller and B. F. Miller. This Journal **113:** 611, 1935.
7. Van, Slyke, D. D., A. Hiller and B. F. Miller. This Journal **113:** 629, 1935.
8. Richards, A. N. Trans. Assn. Am. Phys. **44:** 64, 1929.
9. Lucké, B. The Military Surgeon **99:** 371, 1946.
10. Badenoch, W. W. and E. M. Darmady. J. Path and Bact. **59:** 79, 1947.
11. Richards, A. N., B. B. Westfall and P. A. Bott. Proc. Soc. Exp. Biol. Med. **32:** 73, 1934.
12. Richards, A. N., B. B. Westfall and P. A. Bott. J. Biol. Chem. **116:** 749, 1936.
13. Smith, H. W., W. Goldring and H. Chasis. J. Clin. Inv. **17:** 263, 1938.
14. Finkelstein, N., L. M. Alimosa and H. W. Smith. This Journal **133:** 276, 1941; Smith, H. W., N. Finkelstein, L. Alimosa, B. Crawford and M. Graber. J. Clin. Inv. **24:** 388, 1945.
15. Wearn, J. T. and A. N. Richards. This Journal **71:** 209, 1924; Richards, A. N., J. Bordley, 3rd, A. M. Walker and J. Reisinger. J. Biol. Chem. **101:** 179, 193, 223, 239, 1933.
16. Chesley, L. C. J. Clin. Inv. **16:** 653, 1937; **17:** 19, 1938.

TRAUMATIC SHOCK XVI: AMINO ACID METABOLISM IN HEMORRHAGIC SHOCK IN THE DOG[1]

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A previous communication in this series (1) dealt with the possible relationship between derangements of intermediary carbohydrate metabolism and the progressive deterioration in traumatic shock. In this communication we present analogous data on intermediary protein metabolism in this disorder.

The rise in total blood amino nitrogen in traumatic shock (1–4) has been attributed to impairment of the deaminating function of the liver (2, 3) and to increased production of amino acids because of accelerated tissue breakdown (5). Defective deamination was inferred from the finding of decreased urea synthesis by liver slices taken from shocked rats (6) and the lack of sufficient urea accumulation in the blood of such animals subjected to nephrectomy followed by amino acid administration (7). Defective liver function in shock has been demonstrated (8, 9). Our objective is to identify the nature of the defect in liver function which is specifically related to the fatal character of the shock syndrome. If defective deamination occurs in shock, it is essential to our purpose to determine whether it is causally related to the progressive deterioration in this condition. If the rise in blood amino nitrogen consequent to shock is referable to failure of deamination, the administration of additional amino acid during shock to an animal already laboring under a defective deaminating mechanism should result in abnormal persistence of the amino acid in the circulation. Furthermore, if these abnormalities are intimately related to irreversibility of shock, they should not be favorably influenced by any therapeutic procedure which fails to result in survival. This report is concerned with experiments designed to test the foregoing concepts. The data indicate that *a*) no correlation exists between the development of irreversibility to transfusion and the ability to clear the blood of injected amino acid and *b*) any delay in clearance of injected amino acid from the blood during shock does not persist after transfusion, whether therapeutically effective or not and regardless of the blood amino-acid nitrogen level.

METHOD

Hemorrhagic shock was produced in morphinized mongrel dogs (8–12 kgm., fasted for only 12–24 hours) by a method previously described (10). Solutions of single amino acids in doses of 100 or 200 mgm. per kilogram were injected intravenously before hemorrhage, during the oligemic phase of shock and following transfusion. The amino acids studied were d,l-alanine, glycine, d,l-cysteine hydrochloride, l-lysine monohydrochloride, l-tyrosine, l-tryptophane, d,l-aspartic acid. A proprietary amino acid mixture (casein hydrolysate fortified with tryptophane-Stearn) was also given in doses of 200 mgm. and 2.0 grams/kgm. The volume of the injected solutions varied from 10 to 100 ml., depending upon the solubility of the amino acid. Solution of tyrosine, tryptophane and aspartic acid was effected with the aid of sodium carbonate.

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

Arterial blood specimens were taken immediately before each injection of amino acid and at 15-minute intervals thereafter for one hour. Blood total amino acid nitrogen[2] was determined by the method of Frame, Russell and Wilhelmi (11), blood lactate by the method of Barker and Summerson (12), pyruvate by the method of Bueding and Wortis (13), urea by the method of Karr (14) and nonprotein nitrogen by the method of Koch and McMeekim (15), blood alanine by the method of Alexander and Seligman (16) and blood glycine by a modification of the method of Alexander, Landwehr and Seligman (17). In a few experiments aspartic acid was determined by the method for alanine (16). By comparison with standards of aspartic acid, this method gave values which were approximate only, but of sufficient accuracy in the presence of large concentrations of aspartic acid to be worthwhile.

RESULTS

Data and results are summarized in table 1 and figures are presented of representative experiments. Detailed analysis of the results for each amino acid follows.

d,l-alanine tolerance tests (seven dogs in irreversible shock). (1) In the *normal state*, i.e., prior to the induction of shock, the injection of alanine in the two dosages used (table 1) increased total blood amino-acid nitrogen 3–5 and 5–10 mgm. per cent, respectively (figs. 1 and 2). In five of seven dogs the maximum increase was immediate and a steady decline occurred thereafter. In two animals the maximum rise did not occur until one hour after alanine injection. After one hour some 50–75 per cent of the increase had been dissipated in four dogs, 100 per cent in one. Alanine nitrogen paralleled the blood amino acid nitrogen except in one instance (fig. 1) in which the latter remained high while the alanine fell to normal. This plus the fact that urea did not increase suggests a transfer of the alanine amino nitrogen to form some other amino acid.

(2) During the *oligemic phase* of hemorrhagic shock, the injection of alanine in six dogs produced a somewhat greater immediate increase in blood amino nitrogen

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[2] The whole blood amino acid nitrogen will correctly reflect shifts in the plasma amino acid nitrogen so long as the hematocrit remains essentially constant. In these shock experiments the hematocrit does not vary significantly. Hemodilution causing a drop in hematocrit from 45 to 35 per cent packed cells, which represents an extreme situation, would effect a drop in blood amino nitrogen from, e.g., 8.7 to 8.1 mgm. per cent, if the ratio (2) of amino acid in red blood cells to that in plasma is taken as 2 for the purpose of this calculation.

TABLE 1. PROTOCOL DATA AND SUMMARY OF RESULTS

AMINO ACID	EXP. NO.	FIG. NO.	REVERSIBLE OR IRREVERSIBLE SHOCK	AMINO ACID DOSE	NORMAL STATE		HEMORRHAGIC SHOCK		AFTER TRANSFUSION	
					Amino acid clearance[1]	Increase in blood level of urea[2]	Amino acid clearance[1]	Increase in blood level of urea[2]	Amino acid clearance[1]	Increase in blood level of urea[2]
				mgm. per kgm.						
d,l-Alanine	1		I	100	good				good	
	2		I	100	poor[3]		fair		poor	
	3	1	I	100	fair		fair		fair	
	6		I	200	fair		good		fair	
	13		I	200	good	++	poor	0	fair	++
	14	2	I	200	fair	+	fair	++	good	++
	15		I	200	poor[3]	0	good	++	good	++
Glycine	4	3	I	100	good		fair		good	
	5		I	100	good		fair		good	
d,l-Cysteine	16		I	100	good	++	good	++	good	++
	17	4	R?	200	good	++	poor	0	good	++
l-Lysine	22	5	R	200	good	0	fair	++	good	++
	26		I	200	good	0	good	++	good	++
l-Tyrosine	18		R	100	fair	++	poor	++	good	++
	19	6	I	200	good	0	poor	++	good	++
	20		I	200	fair		good		fair	
l-Tryptophane	21	7	I	200	good	0	good	++	good	++
	24		I	200	good	++	fair	++	good	++
d,l-Aspartic	7		R	100	good		poor		good	
	8		I	100	good		poor		good	
	10	8	I	100	good		poor		fair	
	11		R	100	good		good		fair	
	12		I	100	good		poor		poor	
Amino Acid Mixture	27	9	I	200	fair	+	fair	0	good	+
	28		I	200	good	++	fair	+	good	++
	29		I	300	good	++	poor	++	fair	+
	30		I	2000	good	0				
	31		I	2000			fair	0		
	32		I	2000			fair	0	fair	0
TOTAL					19 good 6 fair 2 poor	6 0 2 + 7 ++	7 good 11 fair 7 poor	5 0 1 + 10 ++	17 good 8 fair 2 poor	1 0 2 + 12 ++
TOTAL IMPRESSION					good	+	fair	+ to ++	good	++

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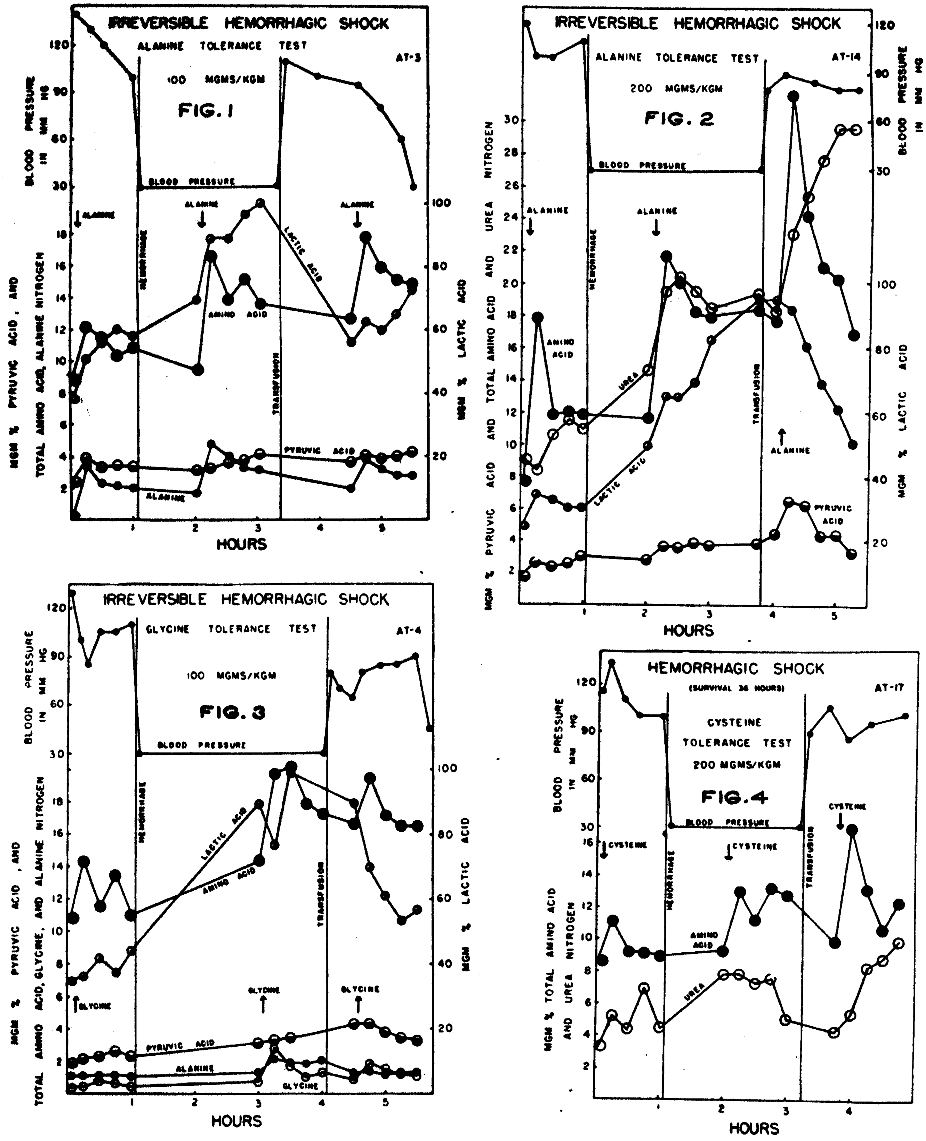
[1] Amino acid clearance as indicated by determination of the total amino acid nitrogen. If the blood amino nitrogen levels one hour after injection were above the starting level by an amount equal to: 0–25% of the total rise, the clearance was considered 'good'; 25–75% of the total rise, the clearance was considered 'fair'; 75–100% of the total rise, the clearance was considered 'poor.'

[2] Urea synthesis was suggested by sharp elevations in blood levels following amino acid injection. In normals the rises in urea were not as high as in shock due to good renal function. Increases in the level of urea nitrogen from the time of amino acid injection are defined as 0 for 0–1 mgm.% rise; + for 1–3 mgms.% rise; ++ for above 3 mgms.% rise.

[3] In these experiments alanine was cleared normally as indicated by alanine determi-

than in the normal state (figs. 1 and 2). This was also true of the blood alanine nitrogen level in the three dogs in which alanine was determined. After one hour, from 50–100 per cent of the increase in blood amino acid nitrogen was still present in four experiments, and none of the increase was present in two. Fifty per cent of the increase in alanine was still present after one hour in all three animals in which alanine was determined.



FIGS. 1–4

Blood urea was studied in three dogs. There was a considerable increase in urea in two dogs (fig. 2). No urea synthesis occurred in the third, in which the increase in the amino acid level persisted during the period of observation.

(3) *After therapeutically ineffective transfusion.* All the foregoing seven dogs proved to be in irreversible shock. Alanine was injected one half to one hour after transfusion. In four dogs the amino acid was administered while the blood pressure was still above 80 mm. Hg; in three dogs the blood pressure was falling rapidly to 35 mm. The increase and decline of the amino acid nitrogen level after alanine administration was practically no different from that in the normal state in two dogs. Clearance was more efficient than in the normal in two others, and slower than in the normal in three, two of which were at a very low hypotensive level and died before the curve could be completed. Of the three dogs in which alanine was determined, two showed the same rate of decline in alanine nitrogen as in the normal state and in one (early death) the decline was slower than in the normal or oligemic phase.

Of three dogs in which urea curves were obtained, two showed some rise in blood urea; in the third animal urea nitrogen increased about 12 mgm. per cent (fig. 2).

(4) *Lactic and pyruvic acids.* Alanine injections caused a moderate rise (10 mgm. per cent) in lactate and a slight rise in pyruvate in the normal state and in two dogs a marked rise in lactic acid and in the lactate/pyruvate (L/P) ratio in the oligemic phase of hemorrhagic shock. The marked characteristic drop in lactic acid and in the L/P ratio following transfusion was followed in three dogs by a secondary rise in lactic acid following injection of alanine (fig. 1).

COMMENT. Alanine was cleared from the blood of the dog in shock somewhat more slowly than in the normal state in some dogs, but as well or even better than in the control state in others, especially after transfusion. The blood amino acid nitrogen level, usually elevated in shock, was only temporarily affected by the alanine. Urea, lactic acid and pyruvic acids were produced following the injection of alanine in the normal state, in the oligemic phase of hemorrhagic shock and following ineffective transfusion.

Glycine tolerance tests (two dogs in irreversible shock). In the *normal state,* both dogs exhibited complete disappearance curves of the increased amino nitrogen following glycine injection. In the first there was very little increase in glycine, but there was evidence of pyruvic and lactic acid formation (fig. 3). In the *oligemic phase* of shock glycine disappeared at the normal rate, but the increase in total amino acid nitrogen level consequent to the administration of glycine diminished at a slower than normal rate. Some alanine was produced (fig. 3). In the posttransfusion phase of irreversible shock the glycine disappearance curve was normal and the lactic acid and L/P ratio dropped precipitously as usual, but the total amino acid nitrogen level did not return to normal.

COMMENT. Despite the fact that injected glycine disappeared from the blood in shock at a normal rate, the elevation in total amino acid nitrogen progressed with the development of shock and continued high after transfusion.

d,l-cysteine tolerance tests (two dogs, one in irreversible shock). One dog was irreversible to transfusion, the other (fig. 4) died in 36 hours (borderline reversibility). In the *normal state* the total amino acid level rose slightly following injection of the amino acid, and as it returned to normal in one hour, blood urea increased. In the *oligemic phase* of shock cysteine clearance was good in one animal and fair in the second (fig. 4). In this dog blood urea remained stationary. After *transfusion* the increase in total amino acid nitrogen level which followed the cysteine injection fell as well as in the normal state. In both dogs urea rose to high levels after amino acid injection, simulating the pattern seen in the normal state.

Comment. During the oligemic phase of shock, elevation in total amino acid nitrogen due to the injection of cysteine was transient, except in one dog. In this instance the failure of urea synthesis and amino acid clearance may be attributed to poor circulation through the liver, since both functions were normal after transfusion. The irreversibly shocked dog showed as good clearance as the dog in reversible shock. The usual rise in total amino acid nitrogen as shock progressed was perhaps less striking in dogs which received cysteine.

l-lysine tolerance tests (two dogs, one reversible and the other irreversible to transfusion). In the *normal state* the injection of lysine was followed by a rise in total amino acid nitrogen in only one animal. In this dog the preinjection level was regained in one hour. Urea did not accumulate significantly, but the nonprotein nitrogen rose seven mgm. per cent. This suggests that lysine was converted to nitrogen-containing compounds which are free of α-amino carboxyl groups and not precipitated with deproteinizing agents (other than urea). In the second dog, no rise in total amino acid nitrogen occurred, but urea was formed. High urea levels were not reached since normal kidney function was still present (fig. 5).

In the *oligemic phase* of shock the injection of lysine was followed by normal amino nitrogen clearance in the first dog and a near normal one in the second. Urea rose in both and nonprotein nitrogen rose in the first.

Following *transfusion* the usually high basal amino acid nitrogen level was not present and the total amino acid nitrogen disappearance curve was normal. Urea production as seen in figure 5 occurred in both dogs. The nonprotein nitrogen rose 20 mgm. per cent above the normal level in the first dog.

Comment. Normal disappearance curves were seen in all phases of shock following injection of lysine in two dogs. Tolerance to injected lysine was the same in irreversible as in reversible shock.

l-tyrosine tolerance tests (three dogs, one reversible and two irreversible to transfusion). In the *normal state* the injection of tyrosine was followed by an immediate rise in total amino acid nitrogen in two dogs. In one of these, return nearly to the preinjection level occurred within one hour (fig. 6). Blood urea remained relatively unchanged. In the other animal the amino acid level was only slightly reduced after one hour. Lactic and pyruvic acid rose 24 and 2 mgm. per cent, respectively. In the third dog, no immediate rise in amino acid followed injection of tyrosine, but a small rise in urea was seen. The nonprotein nitrogen of two dogs did not rise significantly above starting levels.

In the *oligemic phase* of shock amino acid nitrogen rose further following the injection of tyrosine, somewhat belatedly in one animal, steadily from the beginning in the second (fig. 6). In the third, return to preinjection level occurred. Rises in blood urea occurred in the first and second animals. The nonprotein nitrogen rose in the second and third.

Following *transfusion* a normal amino acid tolerance curve was observed in two animals (one in reversible shock and one in irreversible shock), but in the third the curve was only fair, as was the case in this dog in the normal state. The rise in urea was striking in the first and second dogs and the final amino acid level was not significantly elevated.

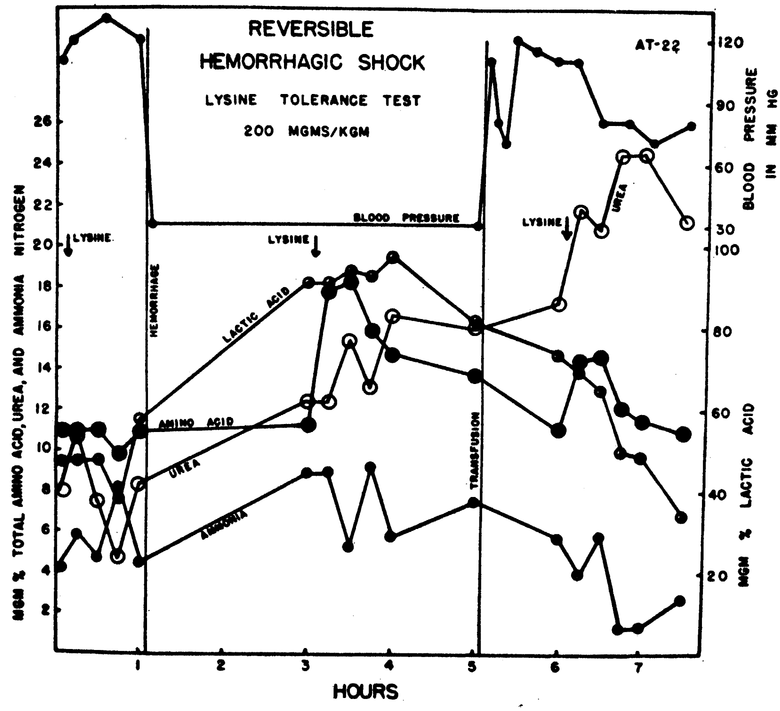


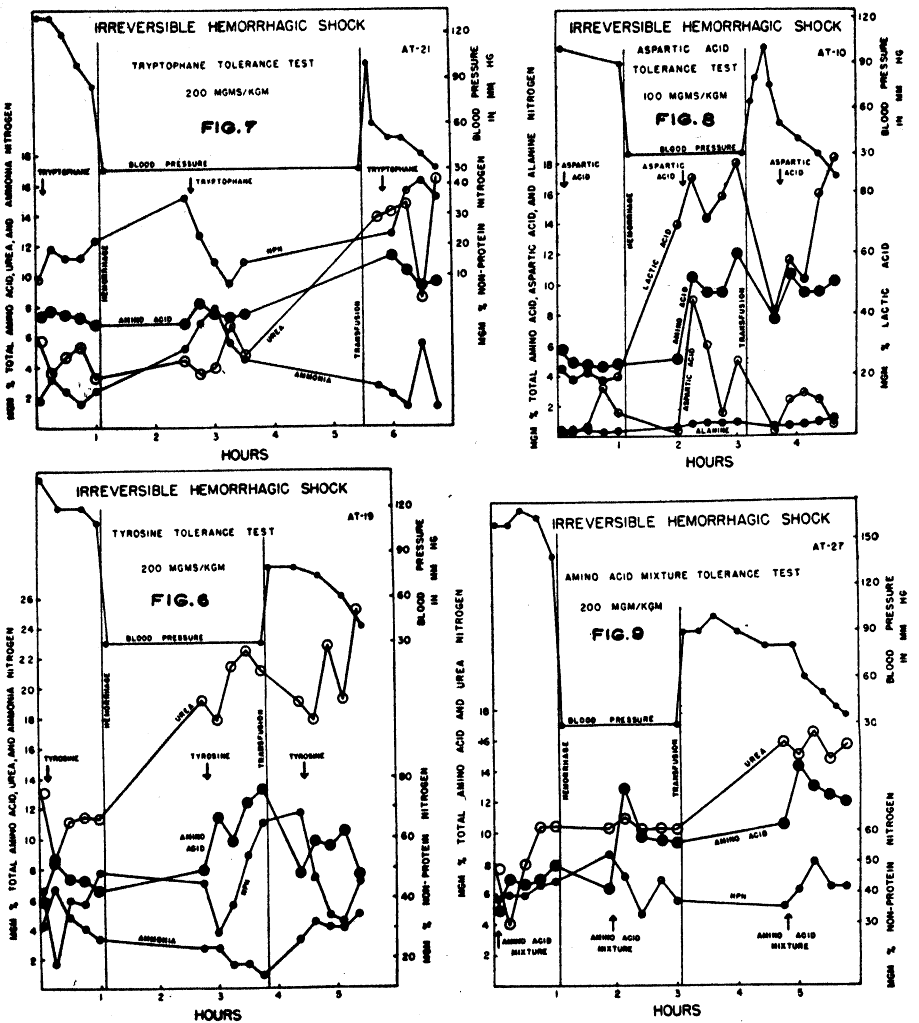
Fig. 5

Comment. Variation in the tolerance curves following injection of tyrosine was noted in three normal dogs. In shock, two of three dogs showed poor blood clearance of amino nitrogen accumulation consequent to tyrosine administration. After transfusion, the clearance was good in all animals irrespective of whether the animals survived or died of shock. No correlation with irreversibility was demonstrable. The usual elevation in blood amino nitrogen associated with shock was not noted in these animals.

l-tryptophane tolerance test (two dogs in irreversible shock). In the *normal state* blood amino acid nitrogen was practically unchanged following the injection of tryptophane, but the nonprotein nitrogen rose, and in one dog urea concentration rose.

In the oligemic phase of shock the amino acid nitrogen rose slightly following the injection of tryptophane and the preinjection levels were reached within an hour. Urea rose after amino acid injection (fig. 7).

After *transfusion* there was good clearance of amino acid nitrogen following rises produced by the injected amino acid. Rises in blood urea were also observed following injection. In both dogs the blood pressure dropped precipitously to about 20 mm. Hg during the last tolerance test. In one the usual rapid drop in blood lactic acid did not occur.



FIGS. 6–9

COMMENT. Normal amino nitrogen disappearance curves following injection of tryptophane were observed in the ~~normal~~ state and in shock before and after transfusion.

d,l-aspartic acid tolerance test (five dogs, two reversible and three irreversible to transfusion). In the *normal state* the injection of aspartic acid did not affect the amino acid nitrogen level materially. In two instances (fig. 8) a drop, and in three a small rise followed by a drop to the preinjection level, occurred within 45 minutes. In one instance a small rise in lactic acid occurred.

In the *oligemic phase* of shock total amino acid nitrogen rose following the administration of aspartic acid, and the change persisted in three animals (irreversible shock). In the fourth (reversible) a slight drop occurred following the rise, and in the fifth (reversible) the aspartic acid was cleared so rapidly that no rise above the usual shock level occurred. Clearance of the aspartic acid as such, determined approximately by a modification of the alanine method (16), occurred during irreversible (two of three instances) and reversible shock (one of two instances). Lactic acid production was observed in two dogs and slight rises in alanine were also noted in one dog.

Following *transfusion* amino acid nitrogen returned to a normal level in one of two dogs in reversible shock in spite of the injection of aspartic acid, but in the three irreversible dogs in which rapid circulatory failure occurred, the amino acid level remained elevated. Good clearance occurred in one of the irreversible dogs, but in the other two in which the clearance was poor and fair in terms of total amino acid nitrogen, clearance of aspartic acid was demonstrated by aspartic acid determinations. Lactic acid production occurred in three dogs following injection of aspartic acid (fig. 8).

COMMENT. Rises in blood amino acid nitrogen resulting from injected aspartic acid persisted during the oligemic phase of hemorrhagic shock (except in one dog that recovered following transfusion). Better clearance of aspartic acid was shown by aspartic acid determination than by total amino nitrogen measurement. This suggests conversion of aspartic acid to another amino acid or fixation of its free carboxyl group so that decarboxylation to acetaldehyde in the determination of aspartic acid was prevented. Aspartic acid clearance was much improved after transfusion, although elevated amino acid nitrogen level persisted. Production of lactic from aspartic acid occurred in all phases of shock. This could occur by deamination and decarboxylation of the free carboxyl group.

Amino acid mixture tolerance tests (six dogs in irreversible shock). In the *normal state* three of four dogs showed good blood disappearance curves in one hour. The fourth exhibited incomplete clearance (fig. 9). How much of the amino acid was incorporated into protein cannot be stated. In one of these dogs (Exp. 30) a very large dose of amino acid mixture (2 grams/kgm.) raised the blood amino nitrogen level ninefold, yet the starting concentration was almost regained at the end of an hour. Since only a slight elevation in urea was observed, it may be concluded that protein synthesis or amino acid storage took place. During the next hour injections of 200 mgm. per kgm. were given at six-minute intervals (this data not included in the table). The amino acid nitrogen level rose steadily to the high level (66 mgm. per cent) which the first single tenfold dose produced. The elevation in blood urea was again comparatively slight.

In the *oligemic phase* of shock the tolerance curve was fair in four and poor in one dog. Urea synthesis occurred in two. In one animal the usual elevation in amino acid nitrogen seen in shock did not occur before or after therapeutically ineffective transfusion. In the two dogs receiving massive doses of amino acid the tolerance curve was fair, but preinjection levels were not reached even two hours later. Urea synthesis was small and out of proportion to what would be expected from the quantity of amino acid cleared. This is interpreted as indicative of protein synthesis or amino acid storage, since urinary excretion of urea or amino acid can be considered negligible under these conditions.

Following *transfusion* good disappearance curves of blood amino nitrogen following administration of the amino acid mixture were obtained in two dogs, despite their irreversibility. The clearance was fair in two dogs. Urea synthesis was noted in three dogs. In one irreversible dog receiving a massive dose of amino acids, amino acid clearance was fair and slow, but urea accumulation was too small to account for the amino acid removed.

Comment. The injection of an amino acid mixture, especially in large doses, before and during shock may be followed by some urea synthesis, but not enough to account for the disappearance of injected amino acid nitrogen. It appears, therefore, that protein synthesis takes place in the normal dog and to some extent in shock.

In the normal animal the injection of amino acid mixtures in repeated fractional doses resulted in accumulation of blood amino nitrogen to levels as high as those reached by a full dose given in one injection. The fact that repeated doses of a certain size and given at certain intervals can result in progressive rise in the level of blood amino acid nitrogen in the normal dog suggests that a steady, increased production of amino acid by tissue breakdown, if great enough (20–30 mgm/kgm/min.), could result in persistently elevated amino acid levels without postulating liver failure.

SUMMARY OF RESULTS

Seven naturally occurring amino acids (three as racemic mixtures) were injected individually into dogs in the normal state during the oligemic phase of hemorrhagic shock and following transfusion. In addition to alanine and glycine, acidic, basic, sulfur-containing and aromatic amino acids were studied, as was also an amino acid mixture. The experiments show that the normal animal does not clear the blood of the various amino acids with equal facility. In hemorrhagic shock the clearance of different amino acids varies even more. In general all the amino acids studied are removed from the blood more efficiently following transfusion than during the oligemic phase of hemorrhagic shock. Even when the transfusion is therapeutically ineffective, there is little or no impairment of the ability to clear the blood of the injected amino acids.

Urea production was observed in all phases of shock as well as in the normal state. The urea levels were not as high in the normal state as in shock, presumably because of good renal function in the former.

DISCUSSION

Although the removal of accumulations of amino acid nitrogen consequent to administration of individual amino acids is to some degree slowed during the oligemic phase of shock, presumably as a result of retarded deamination, the fact that it becomes normal or near normal promptly after transfusion indicates that irreparable damage to the deamination mechanism has not occurred.[3] If deamination were in fact deficient because of injury to enzyme systems, the formation of urea should not occur or should be curtailed. The increases in blood urea which we observed are too great and too sudden to be explained on the basis of decreased renal function. Decreased urea synthesis, reported by Engel (7) following the injection of an amino acid mixture in the oligemic phase of hemorrhagic shock in nephrectomized rats, is not conclusive evidence of loss of the deaminating function since it may reflect a decrease in blood flow through the liver, a vital organ in deaminating activity. The evidence he cites (7) to support his conclusion that there is a true metabolic injury, not just a deficiency in blood flow, is derived from *in vitro* work. Unless the injury he postulates is reversible, his conclusion is contradicted by our observation of recovery of the normal rate of deamination after transfusion, as evidenced by the comparison with the rate of amino nitrogen clearance from the blood in the normal state.

Even though the general trend of protein metabolism in shock is catabolic, protein synthesis may occur. For example, in one experiment in which an amino acid mixture was injected in shock, blood clearance was as good as in the normal state (fig. 9). Although the clearance was slower when ten times as much was injected than with the smaller doses, the amount of urea produced as estimated from blood levels above was too small to account for the amount of amino acid cleared. It is likely that protein synthesis occurred. The negligible rise in urea cannot be attributed to excretion via the kidneys, since no urine is formed at a blood pressure of 30 mm. Hg.

If the poorer blood amino nitrogen clearance noted in the oligemic phase of shock is referable to diminished blood flow, especially in the liver, it is difficult to explain the persistence of a normal rate of clearance after the return of peripheral vascular collapse in the posttransfusion phase. Perhaps even under such conditions the flow of blood through the liver is better than in the oligemic phase. Such flow might be sufficient to maintain the function of deamination, even while the process in the liver which is responsible for progressive vascular failure is not favorably affected; thus death may occur before the return of a slower rate of deamination can be observed. The cross-circulation procedure which corrects a failing peripheral circulation after therapeutically ineffective transfusion requires a substantial interval to reverse the hepatic injury involved (18). All liver functions need not be equally sensitive to the injury from anoxia or equally responsive to therapy.

Why does blood amino nitrogen remain elevated after transfusion even though the injected amino acid may be cleared at a normal rate? Possible explanations are *a*) persistence of normal amino acids so far not studied, *b*) the formation of abnormal amino acids which possess the alpha amino carboxylic acid group and which cannot be metabolized but would be measured by the analytical methods employed, *c*) defective protein catabolism with the release of proteoses or peptides which are not removed by protein precipitation and which might be measured by the analytical methods employed and *d*) accumulation of a nitrogenous nonamino acid substance measured by the relatively nonspecific colorimetric method (11). In support of the last interpretation is the evidence (19, 20) that amino nitrogen accumulation in the blood during shock is less when measured by the ninhydrin gasometric method (21) than by the colorimetric method employed in this investigation (11).

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[3] Although only one fifth of the possible amino acids involved were tested, we believe that the data are applicable to most of the amino acids, since the ones selected may be considered representative of most types. Three of these amino acids were racemic mixtures of the natural and unnatural forms. However, since both forms were deaminated, interpretation of the results remains valid.

In some experiments the injection of cysteine, lysine or tyrosine seemed to suppress the usual progressive rise in total amino acid level in shock. However, in one experiment not reported above, injection of a mixture of lysine, cysteine, tyrosine and tryptophane in irreversible shock failed to lower an elevated amino acid nitrogen level while the injected amino acid nitrogen was cleared from the blood normally.

The accumulation of blood amino nitrogen in shock may be related to disturbance in carbohydrate metabolism because, as has been pointed out in a previous publication (1), the injection of pyruvate in shock lowered the elevated blood amino nitrogen concentration in six of nine experiments (see fig. 11). The result could not be due to transfer of amino nitrogen to pyruvate, but could result from conversion of amino acid nitrogen to ammonia by pyruvate. That this is a possible reaction is suggested by an interesting observation of Greenstein (22). He showed that digests of glutamine with liver extracts yield very little ammonia, but when pyruvate was added, considerable ammonia was produced. He ascribed this to the condensation of glutamine with pyruvate to form the dehydropeptide, gamma glutamyl amino acrylic acid, which, when split by dehydropeptidase, gave glutamic acid, ammonia and pyruvic acid. The pyruvic acid served as a cosubstrate.

Engel (23) reported that in the rat there is a correlation between the level of the plasma amino acid nitrogen and the severity of the shock state, and Kline (24) observed that in the dog there is a correlation between the rate of rise in arterial (plasma) amino acid nitrogen in late shock and survival. From Kline's data it appears that the amino acid nitrogen level merely reflects the progressive disturbance in blood flow as death approaches. He demonstrated that the amino acid output from the limbs, which was considerable early in shock, diminished as shock progressed.

The sharp rise in the arterial plasma amino acid nitrogen in late shock cannot therefore be attributable to greater amino acid production in the periphery. It is more likely due to *decreased overall* amino acid removal by the liver even though more efficient amino nitrogen removal per unit volume of hepatic blood flow was indicated by an increased amino acid difference between portal and hepatic venous blood as shock progressed (24). A decrease in total liver blood flow with concomitant decrease in total amino nitrogen removal by the liver would thus explain rising amino nitrogen in arterial blood during shock despite a diminishing supply from the tissues and efficient amino acid clearance by the liver. This concept is further supported by direct observations showing a marked decrease in liver blood flow in the oligemic phase of shock (25).

Hence the correlation between blood amino acid nitrogen concentration and death or survival in the oligemic phase of shock does not establish a causal relationship between disordered amino acid metabolism and death or survival from shock. We observed no correlation between the capacity to clear amino acid from the blood and the therapeutic effect of transfusion.

The fact that amino acid nitrogen remains elevated for some time after effective therapeutic transfusion as well as in dogs cured by viviperfusion after unsuccessful therapeutic transfusion (18) supports the view that the amino acid level and the mechanism involved in its regulation are not of fundamental importance in the development of irreversibility or in the course of the posttransfusion phase.

CONCLUSIONS

1. Blood disappearance curves for many injected amino acids, singly or in a mixture, were obtained in dogs in the normal state, in hemorrhagic shock and following transfusion. The amino acids studied individually were glycine, alanine, cysteine, lysine, tyrosine, tryptophane and aspartic acid. Normally the injected amino acids were cleared from the blood within one hour in nearly all instances. In hemorrhagic shock some. although not all, dogs showed poor clearance of certain amino acids. Following transfusion clearance was nearly normal in most cases even when circulatory collapse reappeared. There was no correlation between amino acid clearance or blood amino nitrogen concentration and reversibility to transfusion.
2. Rises in blood urea following amino acid injection were observed in some normal dogs and in some shocked animals.
3. The spontaneous rise in total amino acid nitrogen usually seen as shock progressed seemed less in dogs which had received cysteine, lysine or tyrosine.
4. Even when blood amino nitrogen clearance was good in any phase of shock, the increase in total blood amino acid concentration persisted.
5. The injection of large doses of an amino acid mixture resulted in considerable clearance of the blood of the injected amino acids without an equivalent rise in blood urea nitrogen. This would suggest protein synthesis even in dogs in shock.
6. Evidence is presented to show that slower clearance of injected amino acids during the oligemic phase of shock is due to deficient blood flow and not necessarily to damage to deaminase systems. The ability to clear the blood of injected amino acid was not found to bear a significant relationship to the effectiveness of transfusion or to death or survival.

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REFERENCES

1. Seligman, A. M., H. A. Frank, B. Alexander and J. Fine. J. Clin. Invest. **26**: 536, 1947.
2. Engel, F. L., M. G. Winton and C. N. H. Long. J. Exp. Med. **77**: 397, 1943.

3. Russell, J. A., C. N. H. Long and F. L. Engel. J. Exp. Med. **79**: 1, 1944.
4. Engel, F. L., H. C. Harrison and C. N. H. Long. J. Exp. Med. **79**: 9, 1944.
5. Russell, J. A. and C. N. H. Long. This Journal **147**: 175, 1946.
6. Wilhelmi, A. E., J. A. Russell, M. G. Engel and C. N. H. Long. This Journal **144**: 674, 1945.
7. Engel, F. L. and M. G. Engel. This Journal **147**: 165, 1946.
8. Goldberg, M. and J. Fine. Unpublished data.
9. Ebert, R. V., E. A. Stead, J. V. Warren and W. Watts. This Journal **136**: 299, 1942.
10. Frank, H. A., A. M. Seligman and J. Fine. J. Clin. Invest. **24**: 435, 1945.
11. Frame, E. G., J. A. Russell and A. E. Wilhelmi. J. Biol. Chem. **149**: 255, 1943.
12. Barker, S. B. and W. H. Summerson. J. Biol. Chem. **138**: 535, 1941.
13. Bueding, E. and H. Wortis. J. Biol. Chem. **133**: 585, 1940.
14. Karr, W. G. J. Lab. Clin. Med. **9**: 329, 1924.
15. Koch, F. C. and T. L. McMeekin. J. Am. Chem. Soc. **46**: 2066, 1924.
16. Alexander, B. and A. M. Seligman. J. Biol. Chem. **159**: 9, 1945.
17. Alexander, B., G. Landwehr and A. M. Seligman. J. Biol. Chem. **160**: 51, 1945.
18. Seligman, A. M., H. A. Frank and J. Fine. J. Clin. Invest. **26**: 530, 1947.
19. Hoar, W. S. and R. E. Haist. J. Biol. Chem. **154**: 331, 1944.
20. Levenson, S. M., M. A. Adams, R. W. Green, C. C. Lund and F. H. L. Taylor. N. England J. of Med. **235**: 467, 1946.
21. Van Slyke, D. D., R. T. Dillon, D. A. MacFadyen and P. Hamilton. J. Biol. Chem. **141**: 627, 1941.
22. Goncalves, J. M. and J. P. Greenstein. J. Natl. Cancer Inst. **7**: 269, 1947.
23. Engel, F. L. J. Mt. Sinai Hosp. **12**: 152, 1945.
24. Kline, D. L. This Journal **146**: 654, 1946.
25. Blalock, A. and S. Levy. This Journal **118**: 734, 1937.

INFLUENCE OF HYPOTENSION ON CORONARY BLOOD FLOW, CARDIAC WORK AND CARDIAC EFFICIENCY[1]

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The effect of a sudden reduction in systemic blood pressure upon coronary blood flow and the functional capacity of the heart is a question of considerable practical importance. Coronary blood flow and arterial blood pressure have frequently been shown to be closely related; our own studies have confirmed this (1). Also a diminution of cardiac output usually accompanies a hypotension (2–6). We are not aware, however, of studies that have attempted to correlate the changes caused by hypotension in coronary blood flow and cardiac work, nor has the effect of such changes upon the cardiac capacity to do work been investigated.

A series of studies of the coronary circulation and the oxygen consumption of the dog's heart made during the past two years (1, 7, 8) has led recently to the development of a method for the measurement of these functions in animals subjected to no abnormality other than light anesthesia (9). By the use of this method, changes in coronary blood flow and cardiac oxygen metabolism can be studied under conditions approaching normal. Observations made during hypotension under such circumstances should reveal changes approximating those that occur in man. The present investigation was undertaken, therefore, to determine the circulatory and oxygen metabolic adjustments of the heart during hypotension.

METHODS

The methods employed in this investigation were the same as reported previously (9). Dogs, weighing 15 to 30 kgm., were anesthetized with pentobarbital sodium intravenously, 30 mgm. per kgm. with 30 mgm. supplements if needed. Anesthesia was maintained in a light plane. Blood samples were collected from *a*) the coronary sinus through a radio-opaque catheter (#8F or #9F) introduced into the right external jugular vein through a small incision and guided into the coronary sinus under the fluoroscope; *b*) the pulmonary conus by means of a smaller catheter (#6F) similarly introduced; and *c*) a femoral artery by means of an 18-gauge needle inserted into the artery. Each of these was connected to

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a manifold (10); the arterial system was connected in addition to a mercury manometer for the registration of blood pressure.

Coronary blood flow was measured by the nitrous oxide method (10, 11) as applied to the coronary circulation (9, 12). The inhaled mixture consisted of nitrous oxide 15 per cent, oxygen 21 per cent and nitrogen 64 per cent. The period of inhalation was 10 minutes. Cardiac oxygen consumption was calculated from the coronary arterio-venous oxygen difference and the measured coronary flow. The procedures employed in collecting, handling and analyzing the various blood samples have already been described in detail (9, 10, 11).

Cardiac output was estimated by the direct Fick method. The oxygen consumption was calculated from the measurement of the volume of expired air and the analysis of its gas content by the microtechnic of Scholander (13). The expired air was collected by diverting it with flutter valves attached to an endotracheal tube (with an inflated balloon) into a calibrated Tissot spirometer. The period of collection was 5 to 10 minutes, depending upon the tidal exchange.

Peripheral resistance was calculated according to Poisseuilles Law by the standard formula (14):

$$\text{TPR} = \frac{\text{mean aortic pressure (mm. Hg)} \times 1332}{\text{cardiac output in cc. per sec.}} = \text{Absolute Units}$$

Cardiac work was calculated by the formula $W = PQ$ where P is the mean aortic pressure and Q is the cardiac output. The result is expressed as kgm.M/min. The kinetic energy factor has not been considered (15).

Cardiac efficiency was taken to be the ratio between mechanical work and oxygen consumed, both in heat equivalents. It was calculated as in our earlier experiments (7).

Hypotension was produced by the intravenous injection of tetraethyl ammonium chloride in two experiments and by the subdural injection of procaine hydrochloride in six instances. The procaine was injected through a 19-gauge needle introduced into the subarachnoid space at the second or third lumbar interspace. The drug was dissolved in 2 cc. of sterile saline.

RESULTS

The observations made during the control period in 11 dogs are summarized in table 1. In four experiments (14, 16, 21, and 22) the animals had severe anemia and cannot be regarded as normal; it is noteworthy that in three of these coronary blood flow was unusually rapid. Cardiac work tended to be elevated in all cases, chiefly because the cardiac output was high (average 4.2 L/sq. meter/min.). The values for cardiac efficiency were higher than those generally appearing in the literature, but this is not remarkable in view of the figures for cardiac output.

The effects of hypotension are summarized in table 2. The amounts of the drugs used to produce the hypotension are also indicated. In the experiments in which subdural procaine was used, there was flaccid paralysis of the hind limbs from which recovery was complete in all but one instance (expt. 14). Autopsy revealed that the spinal cord of this dog had been injured by the needle, a probable factor in the very low blood pressure which made impossible the collection of a second set of blood samples. There was no way of knowing the level of spinal anesthesia in the other five dogs.

Among the various findings presented in table 2, the following deserve comment. The effect of hypotension on coronary blood flow was uniformly in the direction of a decrease, but in some cases (expts. 16, 18, and 19) the alteration was too small to be significant despite the fact that mean blood pressure and cardiac output had diminished considerably. In three other experiments (8, 21, and 22) in which the changes in coronary flow were large, the decreases in blood pressure and cardiac output were correspondingly great.

TABLE 1

EXPT.	WGT. KGM.	COR. FLOW CC/100 G/MIN.	O_2 CONSUMP. CC/100 G/MIN.	OXYGEN VOL.%			MABP MM. HG	CARD. RATE	C.O. CC/MIN.	CARD INDEX	LF. VENT. WORK KGM/MIN.	EFF. %
				Art.	R. Vent.	Sinus						
6	22.9	79	10.6	17.1	14.8	3.7	125	168	5450	6.05	9.5	32.0
8	21.5	63	8.4	15.4	11.2	2.2	140	132	2960	3.42	5.75	33.7
9	24.8	45	5.6	18.3	15.7	5.9	120	170	2040	2.14	3.40	37.0
10	25.2	107	14.4	16.9	13.5	3.4	176	180	4900	5.10	11.95	36.9
12	30.2	98	13.0	16.5	12.4	3.3	132	124	3860	3.55	7.06	19.4
14	20.9	85	7.4	10.4	6.9	1.7	136	144	3740	4.40	7.10	56.1
16	15.0	105	10.7	11.9	9.0	1.7	95	136	2660	3.9	3.50	19.7
19	15.7	72	10.0	17.2	13.7	3.3	121	216	2830	3.94	4.76	37.7
20	17.2	56	6.8	15.8	11.2	3.7	118	150	3170	4.25	5.10	54.0
21	16.1	139	11.1	10.2	7.0	2.2	120	180	3000	4.22	5.0	27.7
22	19.1	136	13.7	11.4	7.7	1.3	120	150	4570	5.71	7.6	34.6

Cor. flow = coronary blood flow in cc/100 g. lf. ventricle/minute. O_2 consump. = oxygen consumption in cc/100 g. lf. ventricle/minute. Art. = arterial. R. vent. = right ventricle. Sinus = coronary sinus. MABP = mean arterial blood pressure. Card. rate = cardiac rate. C. O. = cardiac output in cc/minute. Card. index = cardiac output in liters/sq. meter of body surface area/minute. Lf. vent. work = left ventricular work in kgm.M/minute. Eff. % = cardiac efficiency.

Left ventricular oxygen consumption varied directly with coronary flow. This was to be expected since the oxygen content of coronary venous blood was already so low in the control period that a significant decrease in the volume of blood flow could not be effectively compensated for by a further widening of the arteriovenous oxygen difference (16). In the experiment in which coronary blood flow decreased least (#19), such compensation proved adequate and the oxygen consumption was not reduced.

Cardiac efficiency was decreased during the hypotension in every case. The correlations of cardiac efficiency with the factors entering into its derivation were: with cardiac output, $r = 0.65$, $p > 0.001$; with cardiac work, $r = 0.61$, $p > 0.001$; with mean blood pressure, $r = 0.52$, $p > 0.01$. Cardiac output and mean blood pressure showed a poor correlation, $r = 0.39$, $p > 0.05$.

Hypotension was not associated with a constant directional change in peripheral resistance. In five experiments (5, 8, 14, 18, and 21) the resistance decreased while in the three others it increased.

TABLE 2

EXPT.	WGT. KGM.	TIME	COR. FLOW cc/100 G/MIN.	O_2 CONSUMP. cc/100 G/MIN.	OXYGEN VOL. %			MABP MM. Hg	CARD. RATE	C.O. cc/MIN.	CARD. INDEX	PERIPH. RESIST.	LF. VENT. WORK KGM/MIN.	EFF. %
					Art.	R. Vent.	Sinus							
5	20.0	12:35	60	8.0	18.1	11.8	4.8	108	155	1460	1.77	5900	2.20	13.7
		1:35	Procaine 40 mgm subdural											
		1:45	53	7.74	17.1	12.1	2.5	85	140	1490	1.81	4550	1.76	11.4
8	21.5	10:15	63	8.40	15.4	11.2	2.2	140	132	2960	3.42	3780	5.75	33.7
		10:45	Procaine 20 mgm subdural											
		11:00	34	3.60	12.2	7.1	1.7	73	130	1690	1.95	3440	1.71	22.9
14	20.9	11:05	85	7.40	10.4	6.9	1.7	136	144	3740	4.40	2910	7.10	56.1
		11:45	Procaine 40 mgm subdural											
		11:55			10.5	5.0	1.2	46	150	1780	2.09	2060	1.1	
16	15.0	10:50	105	10.7	11.9	9.0	1.7	95	136	2660	3.90	2850	3.5	19.7
		11:30	Procaine 20 mgm subdural											
		11:50	98	9.4	10.7	5.6	1.3	72	182	1540	2.26	3750	1.54	10.1
18	18.2	11:10	72	11.3	17.9	11.6	2.1	88	168	1770	2.29	5060	2.16	10.7
		12:05	Etamon 40 mgm intravenously											
		12:10	67	10.1	17.0	9.0	1.8	71	174	1210	1.52	3980	1.20	7.15
19	15.7	10:50	72	10.0	17.2	13.7	3.3	121	216	2830	3.94	3350	4.76	37.7
		11:45	Procaine 30 mgm subdural											
		11:55	69	10.2	16.6	11.0	1.8	95	168	1370	1.91	5540	1.81	13.8
21	16.1	10:50	139	11.1	10.2	7.0	2.2	120	180	3000	4.22	3200	5.0	27.7
		11:30	Procaine 30 mgm subdural											
		11:45	56	4.83	9.7	5.3	1.1	70	115	1840	2.59	3040	1.80	22.7
22	19.1	10:15	136	13.67	11.4	7.7	1.3	120	150	4570	5.70	2110	7.60	34.6
		11:00	Etamon 76 mgm intravenously											
		11:05	85	8.24	10.8	6.2	1.1	89	120	3100	3.88	2290	3.83	19.8

See table 1 for explanation.

To aid in evaluating these findings it was necessary to know the effects of the withdrawal of the amount of blood required for the analyses (for each nitrous oxide determination 65 cc., for each cardiac output determination 15 cc.). This was done satisfactorily in two experiments which are summarized in table 3. While mean arterial blood pressure did not fall appreciably in either experiment, cardiac output declined in both, though more rapidly in one than in the other. Coronary flow changed slightly between the first and second determinations but it diminished considerably by the time of the third. Cardiac oxygen consumption followed the same course as coronary blood flow. Cardiac efficiency in these experiments, however, did not follow either the cardiac work or the cardiac output but tended to remain constant between the second and third periods; in experiment 12 it actually did not change significantly at any time.

The effect on cardiac output and cardiac efficiency of the experimental procedure per se, irrespective of the hypotensive measures, must be kept in mind as one interprets the results. These control observations may indicate that the hypotensive alterations were exaggerated.

TABLE 3

EXPT.	WGT. KGM.	TIME	COR. FLOW cc/100 G/MIN.	O_2 CONSUMP. cc/100 G/MIN.	OXYGEN VOL. %			MABP MM. HG	CARD. RATE	C.O. cc/MIN.	CARD. INDEX	PERIPH. RESIST.	LF. VENT. WORK KGM/MIN.	EFF. %
					Art.	R. Vent.	Sinus							
10	25.2	11:00	107	14.40	16.9	13.5	3.40	176	180	4900	5.1	2860	12.0	36.9
		11:50	112	15.30	16.8	12.1	3.07	172	180	3180	3.3	4310	7.5	24.9
		12:35	78	10.10	15.7	9.3	2.83	165	180	2320	2.4	5680	5.3	26.0
12	30.2	11:00	98	13.00	16.5	12.4	3.26	132	124	3860	3.6	2690	7.1	19.4
		11:50	80	12.40	18.8	14.5	3.31	135	182	3380	3.1	3210	6.3	17.7
		12:30	69	10.60	18.7	12.8	3.0	124	160	3140	2.9	3200	5.4	18.3

See table 1 for explanation.

DISCUSSION

Before one can discuss the effect of hypotension on coronary blood flow and oxygen metabolism, it is necessary to stress again that the values for cardiac output, work and efficiency were unusually high in these experiments. The latter two factors depend directly on the first factor and this was indeed high when compared with the values reported by some other investigators (see Wiggers, 14). However, we have made every attempt to make the results reliable. The fact that the mixed venous blood was obtained from the pulmonary conus and that the catheter was not moved between determinations should obviate most of the usual sources of error (17). Careful checks of the system for measuring and analyzing the expired air have revealed no flaws. We therefore believe our results to be reliable.

The most probable reason for the cardiac output being high is the pentobarbital anesthesia, which is known to have the following effects on the cardiovascular system: *a*) tachycardia due to depression of vagal influences on the heart (18, 19); *b*) peripheral vasodilatation (20, 21); and *c*) a rise in mean arterial blood pressure due to an elevated diastolic pressure (20). If general vasodilatation is accompanied by a raised mean arterial blood pressure, cardiac output must be increased. An increased cardiac output and mean blood pressure imply an increased cardiac work. Bazett (22) considers the cardiovascular changes associated with pentobarbital anesthesia similar to those occurring in an animal engaged in mild muscular exercise. The relatively high values for cardiac output, work and efficiency reported herein when viewed in this light are therefore not remarkable.

With regard to the original purposes of these experiments, one of which was to afford an insight into the effects of hypotension upon the functional capacity of the heart, we have been impeded by the limitations of the criteria available for estimating this factor quantitatively. Coronary blood flow was decreased during hypotension in every case, the average being 25 per cent. At the same time, cardiac efficiency declined just as consistently, the average being 36 per cent. However, it would be wrong to conclude from this that the decrease in coronary flow was responsible for a deterioration in the functional capacity of the heart. This is evident from the fact that cardiac work was reduced an average of 56 per cent because mean blood pressure diminished 29 per cent and cardiac output 42 per cent. The oxygen requirements, in terms of work to be done, therefore were reduced to a much greater degree than the oxygen supply (determined by the coronary flow). We have already demonstrated (7) that the oxygen requirement of the heart is one of the principal determinants of coronary flow. Since in the hypotensive experiments, mean coronary flow decreased only 25 per cent while left ventricular work was reduced 56 per cent, it follows that the coronary flow became more abundant relative to the cardiac needs. The concomitant decrease in efficiency is misleading because it was associated with a relatively excessive coronary flow. However, no other single criterion appears to be better able to indicate the change in the cardiac capacity for work.

The term 'cardiac efficiency' is widely employed as a synonym for the ability of the heart to perform its functions. A brief consideration of the formula used in calculating cardiac efficiency shows that it is not this at all (23). Efficiency here is used in its true mechanical sense referring to the work done by the machine divided by the energy put into it. In the heart, however, oxygen metabolic changes occur which are not associated with the performance of work. These changes are as a result of two factors which are as follows.

(*a*) *'Resting' or 'maintenance' energy utilization.* Any living cell requires a certain amount of free energy simply to maintain its living status, regardless of the nature or degree of its activity. In the case of nerve cells, which do no work (measurable in physical or chemical units), there is at present no means for separating this category of energy utilization (oxygen consumption) from that involved in the performance of a specific function, although the fact that cerebral oxygen consumption runs parallel with cerebral functional activity in animals (24) and in man (11) indicates that some such separation exists. In the heart the situation is somewhat clearer but again there is no separation of the two categories of energy utilization. The significance of this may be gained from figure 1 which summarizes our findings for left ventricular oxygen consumption and left ventricular work in 162 observations representing 49 experiments by either the bubble flowmeter or coronary sinus catheterization technics. It is notable that in no instance did left ventricular oxygen consumption decrease below 2.87 cc/100G/minute even though left ventricular work fell to the negligible figure of 0.16 kgm.M/minute. This value for oxygen consumption cannot be called 'resting' because the average heart rate was about 140/minute. However, it suggests that a considerable amount of oxygen must be used by the heart solely for 'maintenance', regardless of work done. Variations in cardiac efficiency can therefore be in part dependent upon the proportion of this maintenance oxygen value to the total oxygen consumption (23). If all cardiac work is stopped (e.g., by vagal stimulation) only the maintenance oxygen consumption will take place. Under these conditions, the cardiac efficiency would be nil although the capacity of the heart for work might be great.

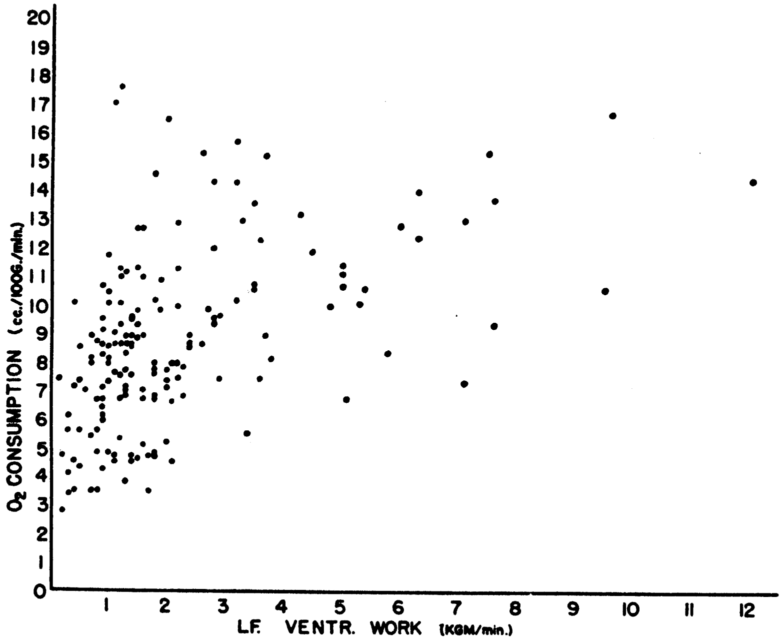

Fig. 1. Ordinate: oxygen consumption in cc/100 grams of left ventrical/minute. Abscissa: left ventricular work in kgm.M/minute.

(*b*) *Energy utilization in the isometric phase of ventricular systole.* The energy expended in ventricular contraction up to the point at which intraventricular pressure equals aortic pressure (isometric contraction) does no mechanical work because no blood is moved. The fraction of the cardiac oxygen consumption dedicated to this part of the cycle should thus vary in the same direction as the aortic pressure. If cardiac work is increased primarily by increasing the blood pressure, a relatively excessive amount of oxygen is needed to do the work and cardiac efficiency decreases. That this actually happens has been demonstrated repeatedly (25, 26, 27, 7). Conversely, if cardiac work is raised primarily by increasing the cardiac output, the oxygen requirement is less for the same amount of work done and the efficiency is improved (25, 26, 27, 7). If aortic pressure were raised so high that intraventricular pressure could not be brought to the same level, no work would be accomplished but the heart would undoubtedly use more oxygen than it would at rest. It is reasonable to assume that the greater the aortic pressure that has to be overcome during the period of isometric contraction, the greater the oxygen metabolism during that period. However, to our knowledge no mathematical method of expression of such relationships has been reported.

Weitz (28) describes Starlings' Law of the Heart (29) as being influenced by, or partly due to, the physical factors that of necessity accompany contraction of circularly arranged fibers. Certainly the relation of the tension of a circular fiber surrounding a sphere to the pressure developed within this sphere is much affected by the radius. However, Weitz accepts the fact that the effect of initial tension on muscular contraction is also involved. No analysis of these complex factors in relation to the work of the heart has been achieved yet to our knowledge.

We believe the conclusion justified that the mechanical conception of efficiency fails to reveal important internal adjustments within the heart muscle during changing conditions.[3] It cannot furnish a valid criterion of the condition of the heart or its ability to meet the demands of the work made upon it, since it indicates only the amount of energy used (oxygen consumed) in ejecting a given amount of blood against a given aortic pressure. Of much greater practical and theoretical importance is an understanding of the factors that determine the oxygen requirements of the heart.

Viewed in this light, the effect of hypotension upon the coronary circulation and heart can be summarized as follows. The decline in blood pressure was associated with a diminution in cardiac output so that total cardiac work was greatly reduced. Coronary blood flow was decreased but not to the degree indicated by the low cardiac work. In these dogs with presumably normal coronary arteries, there was no indication that such a hypotension was harmful to the heart. We do not feel that the decline in cardiac efficiency was an indication that the heart was less able to accomplish the work demanded of it.

SUMMARY

1. In the intact anesthetized dog with coronary blood flow measured by the nitrous oxide method, hypotension was produced by the subdural injection of procaine hydrochloride or by the intravenous injection of Etamon.

2. The fall in blood pressure was associated with a diminished cardiac output in seven out of the eight experiments with a consequent marked reduction in cardiac work.

3. Coronary blood flow decreased in all experiments but remained relatively high in view of the marked decline in cardiac work.

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[3] This would not invalidate statements we have made previously in regard to nikethamide reducing cardiac efficiency (8). In the experiments there cited, nikethamide caused a lowering of the blood pressure, an increased cardiac output and increased cardiac work, all factors that tend to increase cardiac efficiency. In spite of this, the calculated efficiency of the heart was decreased after nikethamide was administered.

4. Cardiac efficiency was reduced in all experiments. There was no evidence that the decline in efficiency indicated that the heart was less able to accomplish the work demanded of it.

5. Evidence is presented that cardiac efficiency is not a valid criterion for indicating the cardiac capacity for work under changing conditions.

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REFERENCES

1. Eckenhoff, J. E., J. H. Hafkenschiel and C. M. Landmesser. This Journal **148:** 582, 1947.
2. Schuberth, O. O. Acta chir. Scandinav. (Supp. 43) **78:** 1, 1936.
3. Rovenstine, E. A., E. M. Papper and S. E. Bradley. Anesth. **3:** 421, 1942.
4. Burch, J. C. and T. R. Harrison. Arch. Surg. **21:** 330, 1930.
5. Goldfarb, W., B. Provisor and H. Koster. Arch. Surg. **39:** 429, 1939.
6. Blalock, A. Arch. Surg. **15:** 762, 1927.
7. Eckenhoff, J. E., J. H. Hafkenschiel, C. M. Landmesser and M. H. Harmel. This Journal **149:** 634, 1947.
8. Eckenhoff, J. E. and J. H. Hafkenschiel. J. Pharm. and Exper. Therap. **91:** 362, 1947.
9. Eckenhoff, J. E., J. H. Hafkenschiel, M. H. Harmel, W. T. Goodale, M. Lubin, R. J. Bing and S. S. Kety. This Journal. In press.
10. Kety, S. S. and C. F. Schmidt. To be published.
11. Kety, S. S. and C. F. Schmidt. This Journal **143:** 53, 1945.
12. Eckenhoff, J. E., J. H. Hafkenschiel, M. H. Harmel, W. T. Goodale, M. Lubin, R. J. Bing and S. S. Kety. Am. J. Med. Sc. **214:** 693, 1947.
13. Scholander, P. F. J. Biol. Chem. **167:** 235, 1947.
14. Wiggers, H. C. This Journal **140:** 519, 1944.
15. Remington, J. W. and W. F. Hamilton. This Journal **150:** 292, 1947.
16. Eckenhoff, J. E. and J. H. Hafkenschiel. Am. Ht. J. In press.
17. Warren, J. V., E. A. Stead and E. S. Brannon. This Journal **145:** 458, 1946.
18. Koppanyi, T., C. R. Linegar and J. M. Dille. Science **82:** 232, 1935.
19. Hafkesbring, R. and W. MacCalmont. J. Pharm. and Exper. Therap. **64:** 43, 1938.
20. Peterson, H. L. and H. C. Bazett. Unpublished data.
21. Goodman, L. and A. Gilman. The Pharmacological Basis of Therapeutics. Macmillan Co., 1941. P. 133.
22. Bazett, H. C. Personal communication.
23. Landowne, M. and L. N. Katz. Medical Physics. Chicago. The Yearbook Publishers, 1944. P. 587.
24. Schmidt, C. F., S. S. Kety and H. H. Pennes. This Journal **143:** 33, 1945.
25. Gollwitzer-Meier, K., K. Kramer and E. Kruger. Arch. f. ges. Physiol. (Pflügers) **237:** 68, 1936.
26. Gremels, H. Arch. f. exper. Path. u. Pharmakol. **169:** 689, 1932.
27. Katz, L. N., E. Lindner and M. Landowne. This Journal **134:** 636, 1941.
28. Weitz, W. Deutsch. Arch. f. Klin. Med. **131:** 47, 1919.
29. Starling, E. H. Linacre Lecture, "The Law of the Heart." London: Longmans, Green & Co., 1915.

ARTERIAL PRESSURE PULSE WAVES IN A PATIENT WITH COARCTATION OF THE AORTA

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Studies of arterial pressure pulse waves in patients with coarctation of the aorta recorded by means of Hamilton intra-arterial manometers in arteries of the upper and lower extremities have clearly indicated that the diastolic pressure is about the same above and below the coarctation, the systolic pressure is higher above the coarctation and that the femoral pulse lags about 0.1 second behind the radial pulse (1, 2). There is no published record of an arterial pressure pulse tracing from a collateral artery which connects the arterial tree above the coarctation directly with that below. Such a tracing was obtained with a Hamilton manometer in a middle-aged white male suffering from coarctation of the aorta.[1] In addition, arterial pressure was recorded simultaneously on the left side in the radial and femoral arteries (fig. 1). It will be noted that the arterial pressure in the radial artery is 215 to 225/100 mm. Hg, in the left collateral subscapular artery 215/100 mm. Hg, in the femoral artery 150/100 mm. Hg. The mean arterial pressure is 104 mm. Hg in the femoral artery, 110 mm. Hg in the radial and collateral arteries and the pulse wave in the femoral artery lags 0.1 second behind that in the radial artery.

Of greater interest than these usual differences is the remarkable similarity between pulse waves from the radial artery and subscapular collateral artery in spite of entirely different anatomical arrangements of the arterial tree distal to the points from which pressure was recorded. At postmortem examination it was shown that the radial arteries were of normal origin and distribution. Enlarged, tortous subscapular arteries whose lumina measured 0.5 cm. joined enlarged fifth and sixth intercostal arteries, the lumina of which measured 0.5 cm. and 0.4 cm., respectively. These latter vessels entered the aorta below the coarctation. The main collateral arteries whose lumina measured 0.7 cm. and 0.5 cm. joined enlarged third and fourth intercostal arteries just below the coarctation. The lumen of the aorta was constricted to 0.5 cm. The ligamentum arteriosum was non-patent.

Since variations in the form of pressure pulse waves are considered to be influenced partly by waves reflected from distal arterioles and to a lesser degree by standing arterial waves (3, 4), the similarity of the waves at the radial and collateral arteries is hard to understand. The pulse wave in the radial artery is of a form generally considered to be more representative of the central pulse but

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[1] These studies were carried out in 1940.

why this is so is not clear. It suggests that local physical conditions in the segments of the arteries from which pressure is recorded are important in determining the contour of the waves and that in this instance they were the same.

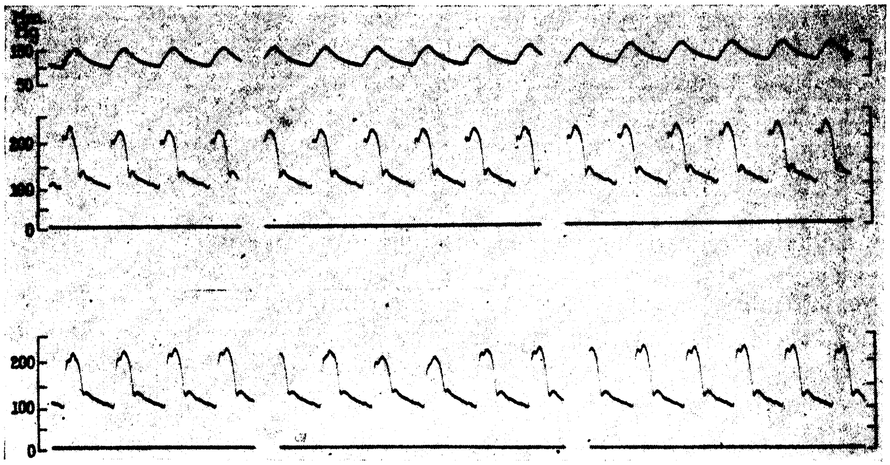

FIG. 1. SIMULTANEOUS RECORDS OF PRESSURE in the left femoral (*upper curve*) and left radial arteries (*middle curve*) taken with Hamilton intra arterial manometers. *Lower curve*, pressure in left subscapular artery recorded a few minutes later.

CASE REPORT

During childhood the patient had occasional convulsive seizures but was otherwise well until the age of 36 (1923) when convulsions began to recur almost weekly, during one of which he developed right hemiplegia and motor aphasia. He was placed in a hospital at the age of 39 (1926) and remained there until his death. On admission to the hospital in 1926, he was noted to have right spastic hemiplegia, Horner's syndrome on the right side, motor aphasia and an arterial pressure of 155/100 in the left upper extremity. Encephalographic studies exhibited atrophy of the left side of the brain and external communicating hydrocephalus. The diagnosis on transfer to Goldwater Memorial Hospital in 1939 was epilepsy, right hemiplegia, and right Horner's syndrome.

It was shortly recognized, by the presence of extensive collateral arteries over the chest and neck and feeble, delayed femoral arterial pulsations compared to pulsations in the radial artery, that the patient was suffering primarily from coarctation of the aorta. The diagnosis was confirmed by angiocardiographic studies[2] which demonstrated a constriction in the aorta at the level of the sixth thoracic vertebra. Roentgenograms of the chest exhibited scalloping of rib margins and enlargement of the heart to the left. Until his death in this hospital, the patient experienced frequent bouts of unexplained fever, convulsive seizures, short periods of incarceration of a left inguinal hernia, hypochromic anemia which responded to iron therapy and suppurative pneumonia in 1945.

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[2] These were performed by Dr. Henry K. Taylor, Chief, X-ray Department, Goldwater Memorial Hospital.

The systolic level of arterial pressure in the upper extremities was generally 180 to 150 mm. Hg and the diastolic 100 to 80 mm. Hg until 1946 when the systolic rose to 190 to 170 mm. Hg and the diastolic fell to 60 to 30 mm. Hg. An aortic diastolic murmur was heard consistently for the first time in 1944. Urea clearance and phenolsulphonephthalein excretion tests of urine function last studied in 1945 were normal. Several cultures of the urine and blood were sterile. In June 1946, he developed heart failure and required digitalis. On February 13, 1947 tonic and clonic convulsive seizures occurred on the left side for the first time and he became unconscious and died. He was 60 years old at death.

Post-mortem examination showed, in addition to coarctation of the aorta and extensive collateral arteries, a congenital aneurysm and compression stenosis of the left middle cerebral artery, extensive encephalomalacia, particularly of the left side of the brain, fusiform aneurysms of the common iliac arteries and a sacular aneurysm (3 cm. diameter) of the abdominal aorta just proximal to its bifurcation.

REFERENCES

1. Woodbury, R. A., E. E. Murphy and W. F. Hamilton. Arch. Int. Med. 65: 752, 1940.
2. Steele, J. Murray. J. Clin. Invest. 20: 473, 1941.
3. Wiggers, C. J. Physiology in Health and Disease. Philadelphia, Lea and Febiger, 1934. Chap. XXXIX.
4. Hamilton, W. F. Medical Physics. Edited by Otto Glasser. Chicago, The Year Book Publishers, Inc., 1944. P. 7.

ARTERIAL HYPERTENSION IN THE CHICKEN[1]

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For some time we have been interested in the production of hypertension in the chicken in order to study the interrelationship between high blood pressure and atherosclerosis. In our preliminary studies on the chicken we attempted to produce hypertension by the Goldblatt principle by ligation or partial constriction of the main renal arteries (1). In some of these animals after partial constriction of the renal arteries on one side, the ureter of the opposite kidney was ligated in order to increase the ratio of ischemic to normal kidney tissue (2). Unilateral and bilateral obstruction of the ureters was also tried. However, none of these measures consistently increased the blood pressure for any significant period. Even when both ureters were occluded and acute bilateral hydronephrosis was produced, a condition which consistently causes a rise in pressure in mammals (3), no increase in pressure was seen in the chickens and the animals died in 12 to 15 hours in acute hyperuricemia (4).

Seyle (5) observed that cardiovascular and renal changes could be produced in chickens by adding NaCl to the drinking water of the animals. The initial changes observed were generalized anasarca, cardiac dilatation and renal alterations resembling subacute glomerulonephritis. Later, dehydration, cardiac hypertrophy and glomerular sclerosis were the outstanding features of this salt intoxication syndrome. These changes suggested the possibility that the animals had developed hypertension, but blood pressures were not reported. The possibility that hypertension had been produced seemed worthy of further investigation. After the present studies were begun, Krakower and Heino (6) published data on the blood pressure of chickens on a salt diet which also suggested that a rise in blood pressure occurred. Our results with methods differing from those of Krakower, but somewhat similar to those of Selye, are presented in this report.

METHODS

Eight white leghorn chicks, six weeks old, were placed on a starting diet of mash and tap water for 16 days. During this period three control blood pressures on each chicken were measured with the Hamilton manometer by means of a small needle inserted into the sciatic artery. The drinking water was then replaced by a 0.9 per cent NaCl solution and the chickens permitted to drink this salt solution ad libitum. Blood pressures were taken every five to eight days. After 33 days, when the rise in pressure was seen to be moderate, the NaCl con-

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[1] Aided by the Sam Ehrlich Memorial Club Fund for Cardiovascular Research.

[2] The Department is supported in part by the Michael Reese Research Foundation.

centration was increased to 1.2 per cent. The salt solution was replaced 26 days later by tap water for 13 days. A second period of salt administration (1.2 per cent) was then instituted for 22 days, during which period blood volume, body weight and hematocrit values were determined in addition to the blood pressures. After a final period of 10 days on tap water the chickens were killed.

RESULTS

The systolic and diastolic blood pressures recorded during the experiment in the eight birds are given in table 1, and the average for the entire group is shown in figure 1. The control blood pressure at the beginning of the experiment averaged 132/117 mm. Hg. The values in the several birds did not vary significantly from this value, which is in the range obtained in our laboratory on a large number of controls (7). The high level of the normal diastolic pressure in the chicken appears to be related to its high normal body temperature of 42°C. (7).

TABLE 1. EFFECT OF SALT INGESTION ON THE BLOOD PRESSURE OF THE CHICKEN

DAY	FLUIDS GIVEN	CHICKEN NUMBER AND BLOOD PRESSURE IN MM.Hg							
		50	51	52	53	54	55	56	57
0	tap water	122/112	120/105	140/125	130/120	133/116	142/120	140/125	125/115
6	“ “	130/119	125/115	150/140	145/135	143/127	142/128	150/135	162/145
12	“ “	122/112	120/110	140/133	140/130	134/129	135/125	133/123	140/130
18	salt 0.9%								
22	“ “	165/155	125/115	145/133	143/133	130/120	125/115	125/115	136/125
29	“ “	158/138	135/125	173/163	155/135	162/142	165/145	160/145	150/135
34	“ “			180/165	145/125	140/133		155/136	155/137
42	“ “	168/150	143/127	175/155	130/115	155/135		152/138	145/128
50	“ “	168/150	150/130	175/160	155/145	145/135		150/140	162/148
51	“ 1.2%								
56	“ “	143/128	170/127	175/153	173/148	135/120		165/148	162/148
62	“ “	183/158	158/134	190/160	180/150			155/138	162/145
69	“ “	178/163	168/138	195/167	165/145			145/130	185/163
76	“ “	195/170	190/150	190/160	158/138			165/145	205/160
77	tap water								
83	“ “	133/120	135/123	135/120				140/125	155/140
89	“ “	137/120	128/115	167/147				133/118	165/145
90	salt 1.2%								
99	“ “	160/145	148/123	170/155				175/157	168/148
109	“ “	165/150	165/150	190/170				175/153	180/160
112	tap water								
121	“ “	136/120	155/145	177/163				180/155	160/138

At the onset of the 0.9 per cent salt administration the blood pressure did not rise significantly, but at the end of 17 days it had risen 22/17 mm. Hg. It then remained unchanged until the salt concentration was increased to 1.2 per cent. This produced a further increase of 25/9 mm. with the average pressure reaching 183/154 mm. Hg. The total rise over the control values was 51/37 mm. Hg. When the salt was discontinued a prompt fall in pressure occurred, averaging

43/28 mm. Hg and bringing the pressure nearly to the control levels. With the readministration of salt (1.2 per cent) in the drinking water, a rise in pressure was again observed, with a fall after the animals were again returned to ordinary tap water. The weight of five birds during the second period of salt intake showed an average decrease of 330 grams, a 20 per cent reduction over a period of 15 days. Within nine days after the salt intake was ended there was an average gain in weight of 40 grams.

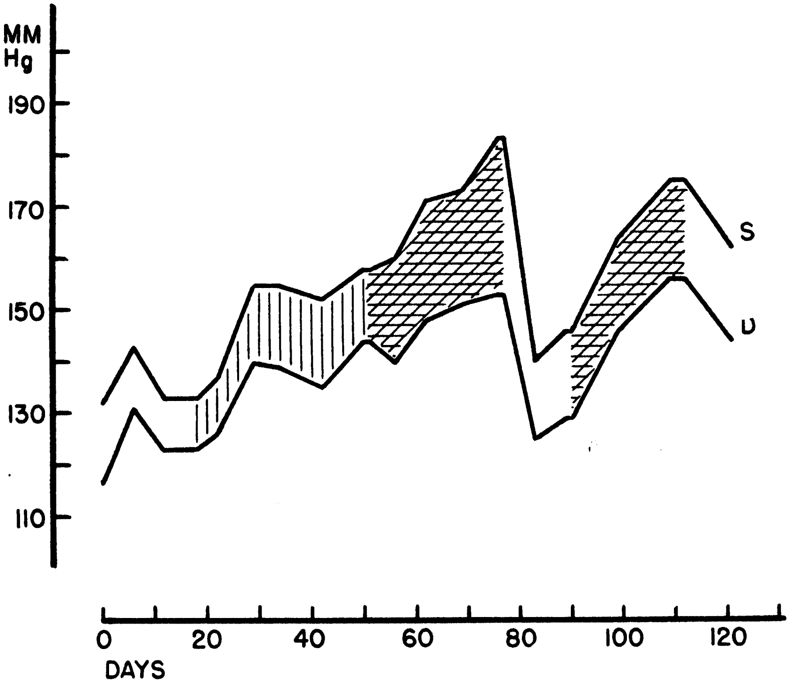

FIG. 1. EFFECT OF INCREASED SALT INGESTION on the average blood pressures of eight chickens. S and D systolic and diastolic blood pressure, respectively. The various periods are indicated by markings between systolic and diastolic pressure lines; periods of tap water ingestion are indicated by blank spaces; 0.9 per cent saline, by vertical-lined period; 1.2 per cent saline, by cross-hatched periods. Ordinates indicate mm. Hg.; abscissae, time in days.

The average hematocrit in these five birds, determined at the same time, showed only a slight increase, from 36 to 39 per cent, during the period of salt administration. Blood volumes measured during the salt intake period in four of these birds showed no consistent deviation from the normal.

The amount of salt solution consumed daily averaged approximately 700 cc. per chicken for the 0.9 per cent salt solution and one liter for the 1.2 per cent concentration. Diarrhea was profuse throughout the period of salt administration. At times the feces were streaked with blood. Obvious anasarca was not observed at any time. No dyspnea was noted. The comb, however, became very pale soon after the salt administration was discontinued. A certain lethargy was also noted while salt was being given.

One bird was found dead on the 16th day of the first salt intake period. It showed no evidence of edema or gross abnormalities except for a small pericardial effusion and slight pulmonary edema. A second bird died 10 days after the salt concentration had been increased to 1.2 per cent. It was distinctly dehydrated; moderate pulmonary edema and a pericarditis with a large effusion and thin pericardial adhesions were present; all organs were markedly hyperemic. A third bird was killed for comparison at the end of the first salt intake period. It was dehydrated, had no pericardial effusion and showed no other gross changes.

Gross autopsy findings in the five birds surviving the four-month period were not remarkable. Two birds had slight pulmonary edema. None had any marked effusions. The pericardium appeared normal in all. One bird had small subepicardial hemorrhages in the region of the left ventricle. No other microscopic changes were found in the heart. Only one bird had a possible cardiac enlargement in comparison to the other birds. The ratio of wet heart weight to body weight ranged between 0.45 and 0.60 per cent. This compares with control values ranging from 0.40 to 0.53 per cent (8). Both Selye (5) and Krakower and Heino (6) found cardiac hypertrophy to be a consistent finding in the salt intoxication syndrome. Krakower's figures are distinctly higher than ours for which differing experiment conditions may be responsible. However, he hesitated to attribute the consistent cardiac hypertrophy to a blood pressure elevation, because the hypertrophy occurred regardless of the presence or absence of hypertension.

None of the birds had contracted kidneys. Histological changes were confined to the kidneys. Variations were apparently related to the time of death. Thus, the bird that died during the early part of the study showed no renal changes of note. The kidneys of the bird dying soon after the salt concentration had been increased to 1.2 per cent showed a moderate proliferation of the glomerular tufts and degenerative changes in the tubules. The most pronounced alterations were found in the kidneys of the chicken killed at the end of the first salt intake period. In this bird, there was a marked proliferation of the glomerular tufts with conspicuous hyperplasia of the individual cells and a moderate proliferation of Bowman's capsule. The proliferated glomerular tufts completely filled the capsular space and the capillaries were compressed and ischemic. The kidneys of the five birds killed at the end of the study showed similar changes but to a lesser degree. Proliferation of glomerular tufts and hyperplasia of Bowman's capsule was still evident, but the capsular spaces were not completely obliterated and the capillaries appeared less compressed. Krakower and Heino (6) found glomerular enlargement to be variable in their salt-treated birds, dependent mainly on the normal rate of growth. Inflammatory or sclerotic changes were not encountered in our series. The tubules were for the most part intact or showed a mild cloudy swelling. Arterial and arteriolar changes were likewise absent. It should be noted that in the normal chicken these vessels present a very well-developed muscular coat.

Gastrointestinal hemorrhages were not found at autopsy in our birds, although they may have been present earlier in the course of the experiment, since blood-streaked excreta were observed in several birds.

DISCUSSION

The intake of 0.9 per cent saline by mammals is generally followed by an immediate transfer of this fluid and salt across the circulatory bed into the interstitial fluid. If a large amount of saline is ingested, it may be greater than can be excreted by the unadapted kidney and thus will lead to the development of edema. This is apparently what occurred in the experiments of Selye (5) and of Krakower and Heino (6). In our experiments, a transient questionable edema developed in some animals but all our animals soon lost weight and actually became dehydrated. This may have been due to an adaptation of the excretory function of the animal to the increased load.

Normally the chicken reabsorbs much of the urinary water in the cloaca and excretes only a paste composed of uric acid and other nitrogenous substances. Under the conditions of our experiments, however, the increased fluid intake occasioned by the high salt content of the water results in an apparent overwhelming of the dehydrating mechanism and in a consequent production of a fluid excreta.

The increase in both systolic and diastolic blood pressures during the course of high salt intake is considered significant because the upward trend was present in all of the birds. By comparison, a control series of blood pressures in five normal young chickens taken over a period of 80 days was found to be remarkably constant; the average variation in systolic and diastolic pressures in this group was 8 mm. Hg, with no tendency for the blood pressure to change significantly during the period.

No less striking was the effect of the return to tap water, which produced an average drop in pressure of 43/28 mm. Hg. Since this phenomenon was observed twice, we feel justified in assuming a direct relationship between high salt intake and the rise in blood pressure in the chicken. The mechanism of this phenomenon is not entirely clear. An increased blood volume is probably not involved as our direct measurements show, and as the marked weight loss and dehydration would seem to indicate.

The production of hypertension in the chicken by a high salt intake in the drinking water appears pertinent to the general problem of the genesis of hypertension. It has been known for some time that desoxycorticosterone, which affects the excretion of Na and K, also produces changes in the blood pressure in man and other animals (cf. 9). Particularly is this true in animals on a high salt diet. The low blood pressures seen in Addisonian patients is also associated with a low plasma sodium concentration, and this hypotension can be treated by the oral administration of sodium chloride (10). Further, the recent emphasis on the use of salt-free diets in the treatment of arterial hypertension (cf. 11), which originated primarily on an empirical basis, may be considered to have some support from these and other findings.

The mode of production of the hypertension in the chicken is not clear at the present time. The proliferative renal changes which are observed may begin as an adaptive response to high salt intake, but finally may act to increase the intrarenal pressure and thus decrease the blood flow through the kidney. The relationship of the sodium chloride to the blood pressure changes would, however, emphasize the possibility that the adrenal cortex has an important rôle in the hypertensive process.

SUMMARY

1. The blood pressures of chickens were measured at frequent intervals before, during and after periods of substitution of 0.9 and 1.2 per cent of saline solution for the drinking water. Some determinations of body weight, hematocrit and blood volume were also obtained.

2. The average systolic and diastolic blood pressures increased progressively during the period of salt administration from an average of 132/117 mm. Hg at the beginning of the increased salt intake to a maximum average value of 182/154 mm. Hg. The blood pressure fell promptly to control levels after the withdrawal of NaCl. Similar observations were made during two periods of salt administration and two periods of return to the drinking of tap water. The degree of hypertension appeared to depend upon the concentration of salt in the ingested water.

3. Hyperplasia and proliferation of glomerular tufts and Bowman's capsule with compression of the capillaries were found after prolonged periods of the substitution of saline for drinking water. This hyperplasia is reversible.

4. The relation between the kidney and factors affecting salt metabolism in the production of this hypertension is discussed.

We are indebted to Dr. O. Saphir of the Pathology Department for his critical review of the microscopic sections.

REFERENCES

1. Goldblatt, H. Ann. Int. Med. **11:** 69, 1937.
2. Katz, L. N., M. Friedman, S. Rodbard and W. Weinstein. Am. Heart J. **17:** 334, 1939.
3. Megibow, R. S., S. Friedberg, S. Rodbard and L. N. Katz. Proc. Soc. Exp. Biol. Med. **43:** 245, 1940.
4. Levine, R., W. Q. Wolfson and R. Lenel. This Journal **151:** 186, 1947.
5. Selye, H. J. Am. Vet. Med. Assoc. **103:** 140, 1943.
6. Krakower, C. A. and H. E. Heino. Arch. Path. **44:** 143, 1947.
7. Rodbard, S. and M. Tolpin. This Journal. In press.
8. Dauber, D. V. and L. N. Katz. Arch. Path. **34:** 937, 1942.
9. Rodbard, S. and S. C. Freed. Endocrinology **30:** 365, 1942.
10. Loeb, R. E. Proc. Soc. Exp. Biol. Med. **30:** 808, 1933.
11. Grollman, A., T. R. Harrison, M. F. Mason, I. Baxter, I. Crampton and F. Reichsman. J. Am. Med. Assoc. **129:** 533, 1945.

VALIDITY OF T-1824 IN PLASMA VOLUME DETERMINATIONS IN THE HUMAN

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Cruickshank and Whitfield (1) in 1945, following experimentation on the cat, stated that a variable portion of initially injected T-1824 was phagocytized by the reticuloendothelial system, thereby 'blocking' that system so that *all* of a second injection of dye would be available for dilution. This concept has since been termed the 'cat effect.' They felt that plasma volume determinations based on single injection dye-dilution techniques would be subject to a possible error up to 20 to 30 per cent. Mather, *et al.* (2) reported the occasional occurrence of the 'cat effect' in humans. Miller (3, 4) experimenting on dogs concluded that the correct interpretation of Cruickshank and Whitfield's results was uncertain. Noble and Gregersen (5) reported that they had never seen the 'cat effect' in dogs or humans studied in their laboratory, although Meneely, *et al.* (6) recently stated that the validity of the blue dye method for estimation of plasma volumes was open to question. It seemed worthwhile to reinvestigate this problem in the human.

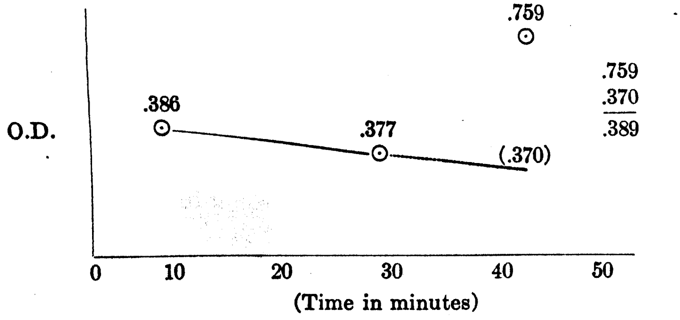
We felt it could be assumed that the plasma volume of normal, basal adults while at rest in bed would not change appreciably over a period of 40 minutes (small fluid shifts could be ascertained and corrected for, by estimations of the serum specific gravity) and that two consecutive estimations of plasma volume by identical techniques, 30 minutes apart, should be the same, within the limits of experimental error. If the 'cat effect' was present, however, the first determination should be appreciably higher (dye concentration of the unknown relatively lower) than the second, which would lead to the assumption that there had been destruction, phagocytosis, or removal of a portion of the initially injected dye. Further, we felt that the use of single 10-minute samples (5) for the calculation of plasma volumes, rather than backward extrapolation of disappearance curves, would be justifiable for two reasons. First, the two independent estimations of plasma volume could be made within a shorter period of time, favoring the maintenance of a 'steady state'. Second, presumably the 'cat effect' appears early on the disappearance curves and, if it exists in the human, the 10-minute samples would show the effect adequately.

Accordingly, six young, healthy, adult males, following at least 6 hours of sleep and 12 hours of fasting, were injected intravenously with T-1824 (lot #L6307) purchased from the Warner Institute. The exact amount was determined by a weighed syringe technique and approximated 20 mgm. in each instance. Control samples were taken immediately before injection, and exactly 10 and 30 minutes after injection samples were again removed. A second weighed injection of approximately 20 mgm. was made about 2 to 4 minutes following withdrawal of the 30-minute sample. Ten minutes after this injection samples were removed. Samples were taken in duplicate from left and right antecubital veins by separate but simultaneous venipunctures. Precautions were taken to avoid hemolysis and stasis. Timing was done with a stopwatch.

TABLE 1. R. S., 12–16–47

Mgm. dye injected: Injection A—— 19.074 mgm.; Injection B—— 19.158 mgm.

	TIME IN MINUTES	ARM	O.D. L620	O.D. L540	O.D. CORRECTED FOR HEMOLYSIS	SERUM SPECIFIC GRAVITY	O.D. CORRECTED FOR HEMOLYSIS AND FLUID SHIFTS
Control	0	L	0	0		(1.0249)	
	0	R	0	0		(1.0249)	
Injection A	0	L					
	10	L	0.382	(.199)	0.383	(1.0247)	0.386
	10	R	0.382	(.196)	0.383	(1.0247)	0.386
	30	L	0.367	(.172)	0.370	(1.0245)	0.376
	30	R	0.370	(.190)	0.372	(1.0245)	0.378
Injection B	32	L					
	42	L	0.739	(.336)	0.743	(1.0244)	0.759
	42	R	0.755	(.381)	0.760	(1.0250)	0.758



$$\text{Plasma volume} = \frac{\text{mgm. injected} \times 0.540 \times 100}{\text{O.D. of unknown}} = \text{Plasma volume A: } \frac{19.074 \times 0.540 \times 100}{.386} = 2670 \text{ ml.}$$

$$\text{Plasma volume B: } \frac{19.158 \times 0.540 \times 100}{.389} = 2661 \text{ ml.} + 15 \text{ ml. (plasma removed)} = 2676 \text{ ml.}$$

Disagreement between A and B: 6 ml. % disagreement between A and B: *0.2%*

The samples were allowed to clot and then centrifuged, the serum being removed and recentrifuged. All samples were read directly against the initial dye-free serum in a Coleman Junior Spectrophotometer using a wave length of 620 mμ. The optical densities obtained were corrected for imperceptible hemolysis by the method used by Price and Longmire (7). Each sample was then corrected for small fluid shifts by utilizing changes in the specific gravity of the serum, assuming that the total plasma protein had remained constant throughout the procedure. (The corrections were obtained by applying the following formula:

$$\text{O.D.}_t \times \frac{\text{S.G.}_0 - 1.007}{\text{S.G.}_t - 1.007} = \text{O.D.}_c$$

in which O.D._t = optical density at time t after the first dye injection; S.G._0 = serum specific gravity of the control sample before the first dye injection; S.G._t = serum specific gravity at time t; O.D._c = theoretical optical density if plasma volume had remained unchanged.) All optical densities, therefore, represent the concentrations of dye that would be present if the plasma volume had not changed during the procedure. The approximate disappearance curve of the initially injected dye, based upon the 10- and 30-minute samples, was projected for another 10 minutes using semi-log paper for the plot. The optical density at this point was subtracted from that of the second 10-minute sample to correct for the residual dye present in the serum and its disappearance from the time the 30-minute samples were withdrawn until the second 10-minute samples were taken. Plasma volumes were then calculated after the method of Noble and Gregersen (5). The volumes calculated from the reinjected dye were also corrected for the plasma removed during the procedure. A representative experiment is illustrated in table 1. Results are summarized in table 2.

TABLE 2

Subject		J. G.		H. T.		K. G.		A. T.		W. W.		R. S.	
Date		12-5		12-9		12-10		12-11		12-12		12-16	
Arm		L	R	L	R	L	R	L	R	L	R	L	R
Optical densities corrected for hemolysis and fluid shifts	Control	0	0	0	0	0	0	0	0	0	0	0	0
	A.10 min.	.302	—	.311	.310	.311	.314	.314	.317	.296	.296	.386	.386
	30 min.	.290	.291	.300	.301	.302	.302	.306	.306	.285	.285	.376	.378
	B.10 min.	.584	.589	.611	.612	.600	.600	.598	.600	.578	.578	.759	.758
Plasma volume A		3188 ml.		3149 ml.		3184 ml.		3417 ml.		3414 ml.		2670 ml.	
Plasma volume B		3213 ml.		3215 ml.		3220 ml.		3374 ml.		3440 ml.		2676 ml.	
Disagreement		25 ml.		66 ml.		36 ml.		43 ml.		26 ml.		6 ml.	
% Disagreement		0.8%		2.1%		1%		1.3%		0.8%		0.2%	

The 'cat effect' was not observed in this series of six consecutive experiments. The close agreement of optical densities of the 10-minute samples, which were taken simultaneously from left and right arms, is further evidence that in the normal, basal human the dye is completely mixed within 10 minutes' time. In our hands the use of T-1824 in the normal, basal human for determination of plasma volume (using a 10-minute sample for calculation of each plasma volume) suggests reliability as shown by the close agreement of optical densities of samples withdrawn simultaneously, and by the checks obtained in repeat estimations of plasma volume within a period of 40 minutes. Although recent work on dogs (4) questions whether the 10-minute sample actually measures *total* plasma volume, our choice of 10-minute samples in these experiments would still appear to be valid, in that, at least, we are attempting to measure the *same* volume of plasma by each reinjection. Our use of a single 10-minute sample was for the reasons outlined above and even though our calculated plasma volumes may represent the 'circulating plasma volume' (4), we feel that the close agreement of the independently calculated plasma volumes (an average difference of 35 ml. in an average plasma volume of 3170 ml.) enhances the probability that the correct use of disappearance curves of the dye T-1824, with backward extrapolation to zero time, will give even more reliable estimates of total plasma volume.

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REFERENCES

1. Cruickshank, E. W. H. and I. C. Whitfield. J. Physiol. **104:** 52, 1945.
2. Mather, K., R. G. Bowler, A. C. Crooke and C. J. O. R. Morris. Brit. J. Exp. Path. **28:** 12, 1947.
3. Miller, A. T. Federation Proc., **5:** 1, 1946.
4. Miller, A. T. This Journal **151:** 1, 1947.
5. Noble, R. P. and M. I. Gregersen. J. Clin. Invest. **25:** 158, 1946.
6. Meneely, G. R., Wells, E. B. and Hahn, P. F. This Journal **148:** 3, 1947.
7. Price, P. B. and W. P. Longmire. Bull. Johns Hopkins Hosp. **71:** 51, 1942.

SERUM AC-GLOBULIN: FORMATION FROM PLASMA AC-GLOBULIN; ROLE IN BLOOD COAGULATION; PARTIAL PURIFICATION; PROPERTIES; AND QUANTITATIVE DETERMINATION[1]

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In work on the purification of prothrombin (1–4) it was noted that thromboplastin and calcium ions activated purified prothrombin less rapidly than prothrombin in native plasma (4, 5). This indicated that either the prothrombin molecule was damaged during the purification procedures or that a 'convertibility factor' had been removed. The possibility of damage to the molecule seemed likely because of the ease with which many ordinary laboratory manipulations destroyed the activity completely. A slight change in reactivity was, therefore, regarded as a logical intermediate stage in denaturation. This belief was further strengthened by the fact that heating not only destroys prothrombin but leaves some material of low-grade reactivity (2). Thrombin produced much the same effect on prothrombin (6), and experiments with fibrinolysin gave typically the same picture (7). As a result of these facts, the term *paraprothrombin* was proposed by Seegers (8) as a convenient description of this sluggish reacting prothrombin (7, 8).

The possibility of the existence of a convertibility factor was indicated in several reports (9–11). The prothrombin conversion rate was found to vary from one species to another being especially slow in man and guinea pig (9). Compensatory mechanisms were found to exist in vitamin K deficiency at low prothrombin levels (10, 11), providing for adequate coagulation by increasing the rate of prothrombin conversion. In one report, Smith reasoned that prothrombin activation rate is "determined by a 'convertibility factor' of unknown nature" (12).

Conclusive evidence for the existence of such a factor was obtained independently in three different laboratories from studies with purified materials (13–16). A human case deficient in the factor was described by Owren (17). The accelerator factor has been obtained in concentrated form in two laboratories (16, 18). When small amounts of the concentrates are added to purified prothrombin products, the prothrombin activation rate is invariably as rapid as that found in native plasma. This seems to preclude the possibility of damage occurring to the prothrombin molecule during purification. Consequently there is now no need for the term paraprothrombin (7, 8). Attention has been directed to studying the convertibility factor. It accelerates the interaction of prothrombin, thromboplastin and calcium ions. Since it is a globulin and an accelerator, it has been referred to by the term 'Ac-globulin' (15). It has been shown by Ware, Murphy and Seegers (19) that a similar, but more potent, accelerator is present in serum. To distinguish between the two it is convenient to speak of plasma Ac-globulin and serum Ac-globulin in accordance with their respective occurrences or origins in plasma or serum.

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The following facts were deduced from experiments to be described in this paper. It is probable that plasma Ac-globulin is a pro-enzyme, therefore, being completely inactive. It is partially activated by amounts of thrombin which are too small to cause clotting of plasma. Slightly larger quantities of thrombin completely activate the enzyme and greater amounts, in addition, actually destroy its activity. The chemical properties of serum Ac-globulin are similar to those of its plasma precursor. The methods described herein for the purification and quantitative determination of serum Ac-globulin are fundamentally similar to those already described for plasma Ac-globulin (18).

EXPERIMENTAL

Relative effects of serum and plasma on the activation of purified prothrombin. Blood collected from a single cow was divided into two parts. One part was allowed to clot and the other was mixed with one seventh its volume of a solution of 1.85 per cent potassium oxalate. An hour later the two parts were centrifuged and the activities of the accelerator substances present in the serum and in the plasma were compared at three different concentrations (fig. 1) by a procedure similar to the two-stage method for determination of prothrombin (20–22). The serum or plasma was allowed to act on prothrombin in the presence of an excess of thromboplastin, under standarized conditions, for varying lengths of time; the quantity of thrombin formed was then measured in terms of clotting time determined by the addition of standarized fibrinogen.

In the same concentrations, serum produced thrombin much more rapidly than plasma. Within the limits of experimental error, the final yields of thrombin were the same in each paired experiment. It is apparent that plasma Ac-globulin becomes more active as a result of the blood coagulation processes. However, it is not indicated whether the accelerator is completely activated in serum.

Activation of plasma Ac-globulin. Thrombin is the substance which produces the change occurring in Ac-globulin during clotting (19). Therefore, it is necessary to study the effects of thrombin on oxalated plasma. The plasma was incubated with varying amounts of thrombin (table 1). The thrombin was a very active concentrate of purity comparable to the products described by Seegers and McGinty (23). It is very improbable that, in the dilutions used, other clotting factors were introduced by the added thrombin. The formation of serum Ac-globulin was studied by diluting the mixtures of plasma and thrombin (table 1) with saline and by incubation with a standarized mixture of prothrombin, thromboplastin and calcium ions. By measuring the time required for the thrombin formed to clot fibrinogen, a quantitative concept of thrombin yield was obtained. The results are recorded in table 1 and in figure 2.

One tenth to 0.2 of a unit of thrombin was usually not sufficient to clot the plasma in one hour, but it was adequate to produce serum Ac-globulin in quantities easily detectable. Ten units of thrombin were apparently required to produce a maximum yield of serum Ac-globulin. With 30 or more units of thrombin the resulting serum Ac-globulin was less active and with 200 units of thrombin the activity was reduced by 90 per cent. This loss of activity is believed to be due to the destruction of serum Ac-globulin by thrombin. Preliminary experiments with purified Ac-globulin and thrombin substantiate that view.

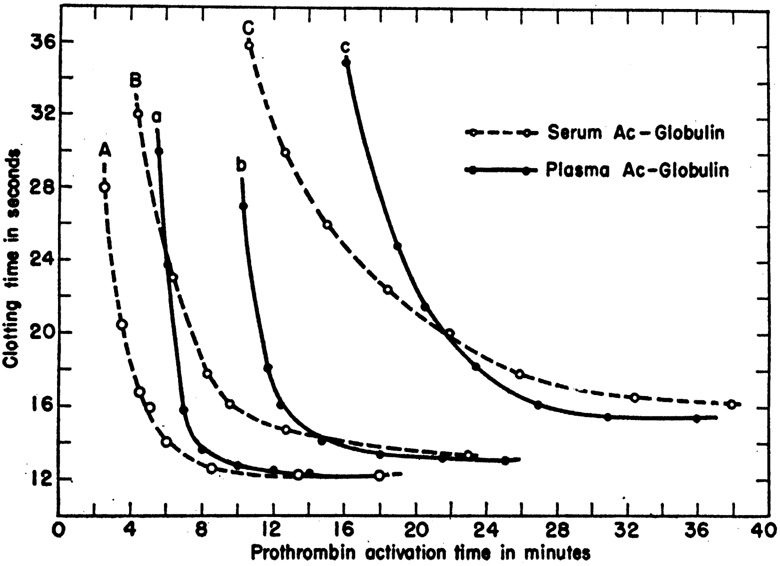

FIG. 1. RELATIVE EFFECTS OF SERUM AND PLASMA ON THE ACTIVATION OF PROTHROMBIN. A and a, serum and plasma, respectively, diluted 1500 times; B and b, serum and plasma, respectively, diluted 4500 times; C and c, serum and plasma, respectively, diluted 20,000 times. Dilutions of plasma include anticoagulant added. The diluted serum or plasma, plus a constant amount of Ac-globulin free prothrombin, were added to the incubation mixture recommended for the two-stage analysis for prothrombin (20–22). Thrombin formation was measured by addition of fibrinogen at intervals. Clotting time is inversely proportional to thrombin concentration.

As a matter of interest it seemed advisable to determine whether fibrinolysin can activate plasma Ac-globulin. In concentrations of 1 to 20 units (8), added to 1 cc. of oxalated plasma, no activation was noted.

Purification of serum Ac-globulin. Work on the purification of serum Ac-globulin was conducted with two objectives in view. One was to find a method for obtaining potent concentrates; another was to test the theory that true serum Ac-globulin is the same as the serum Ac-globulin produced artificially by mixing thrombin and oxalated plasma.

Beef serum was obtained from bovine blood after it had stood at room temperature for five to six hours. Defibrinated oxalated beef plasma was obtained by the addition of Thrombin Topical [Parke, Davis and Co., prepared as described (2)] in a concentration of 15 units per cc. to oxalated plasma.[2] The thrombin was added at room temperature and the fibrin clots were removed as they formed. It was not possible to distinguish between the serum Ac-globulin products obtained from either source. The best yields, with either source material, were obtained by the method already described for preparation of plasma Ac-globulin in concentrated form (18). The method consists of acid fractionation of diluted plasma or serum, adsorption on magnesium hydroxide suspension, elution with carbon dioxide under pressure, fractionation with cold concentrated ammonium sulfate solution, dialysis to remove salts, and isoelectric fractionation.

TABLE 1. INFLUENCE OF VARYING AMOUNTS OF THROMBIN ON THE AC-GLOBULIN IN OXALATED BOVINE PLASMA

REACTING CONSTITUENTS		REACTING TIME	PRODUCTS OF REACTION[3]
Oxalated Plasma	Thrombin		
cc.	*units*[1]	*hours*[2]	
1	0	0	No reaction. Tube contains typical plasma Ac-globulin.
1	0.1 to 0.2	1	Serum Ac-globulin always detected. Usually no clot formed.
1	1	1	Formation of some serum Ac-globulin. Partial clot formation.
1	3	1	Large amount of serum Ac-globulin formed. Clot formed.
1	10	1	Complete conversion to Ac-globulin. Clot formed.
1	30	1	Serum Ac-globulin formed and some destroyed. Clot formed.
1	100	1	Serum Ac-globulin formed and considerable portion destroyed. Clot formed.
1	200	2	Serum Ac-globulin formed and 90% destroyed. Clot formed.

[1] Added in a volume of one cc. to give final concentration of thrombin indicated.

[2] The reaction between plasma Ac-globulin and thrombin to form serum Ac-globulin is rapid. Time was allowed for destruction of added thrombin by anti-thrombin.

[3] If clots were present they were removed mechanically and the resulting Ac-globulin was measured by its ability to accelerate the interaction of prothrombin, thromboplastin and calcium ions (fig. 2).

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[2] The plasma was oxalated with a special oxalate mixture, low in electrolytes (3).

Serum Ac-globulin of high purity has also been obtained by addition of small amounts of thrombin, one unit per cc. or less, to purified plasma Ac-globulin. This method of preparation is preferred because of its simplicity and because the products are of higher quality than those prepared from either serum or defibrinated plasma.

Quantitative analysis of serum Ac-globulin. Quantitative measurement of activity is made by an adaptation of the two-stage method for determining prothrombin (20–22). When the prothrombin, thromboplastin, calcium ions, temperature, salt concentration, pH, etc. are kept constant, the rate of thrombin formation is proportional to the quantity of serum Ac-globulin in the reaction mixture. The curves in figure 3 represent the different rates of thrombin production resulting from varying concentrations of serum Ac-globulin. The curves are similar to those described elsewhere for quantitative determination of the plasma accelerator (18) and they were obtained by the same procedures. In order to determine the concentration of serum Ac-globulin in an unknown, it is first necessary to add a portion of it to the reaction mixture containing standardized prothrombin, thromboplastin and calcium ions. Thrombin formation is

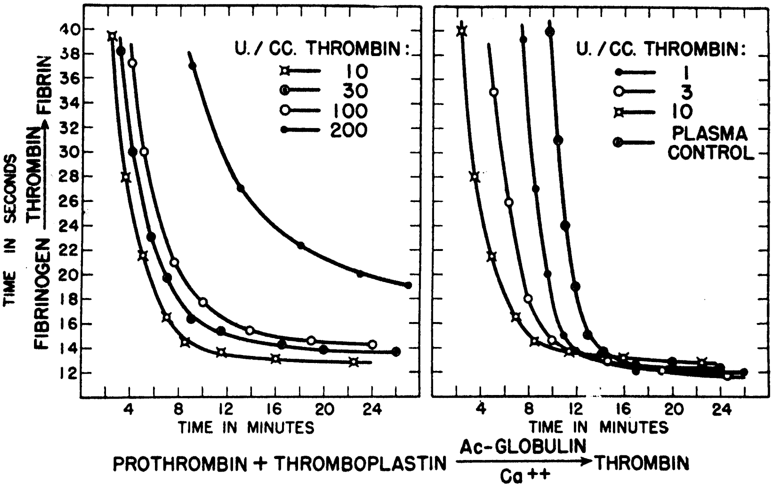

FIG. 2. INFLUENCE OF VARYING AMOUNTS OF THROMBIN ON THE AC-GLOBULIN IN OXALATED PLASMA. The plasma was treated according to the outline in table 1, diluted 5000 times, and allowed to accelerate the activation of standardized prothrombin. Thromboplastin and calcium ions were used according to the specifications of the two-stage analytical procedure for prothrombin (20–22). The thrombin formed in these reactions was measured at intervals by the addition of fibrinogen. The clotting times (ordinate) found at various activation times (abscissa) represent the curves in the figure. Thrombin concentration is inversely proportional to clotting time. The standard prothrombin preparation was Ac-globulin free and in the concentrations used yielded 12-second clotting with fibrinogen when fully activated to thrombin. The curves at the right represent various stages in the formation of serum Ac-globulin from plasma Ac-globulin; those at the left indicate that serum Ac-globulin was formed and partially destroyed by relatively large amounts of thrombin.

then followed by addition of fibrinogen, at intervals, to portions of the reaction mixture. By plotting the activation curve on figure 3, the concentration of serum Ac-globulin in the unknown can be estimated. If the activation curve of the unknown does not fall between curves 5 and 60 on the figure, a new dilution of unknown should be made because this range has been found to be the most accurate and reproducible portion of the family of curves.

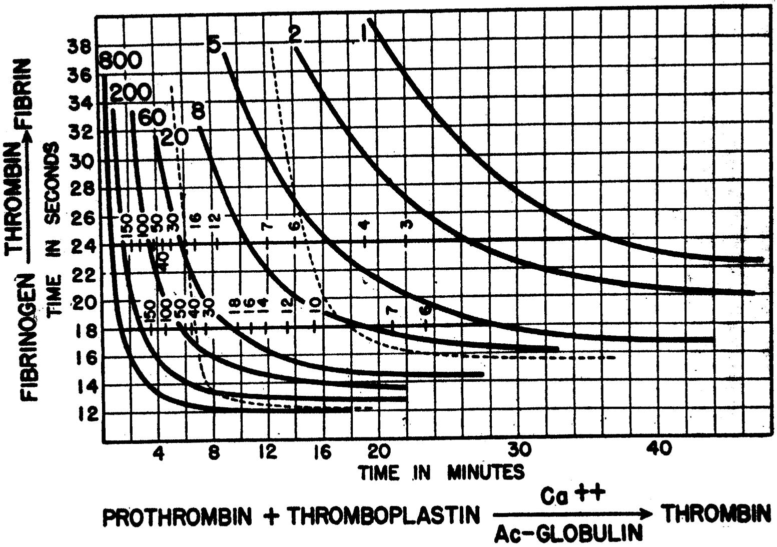

FIG. 3. ACCELERATION OF PROTHROMBIN ACTIVATION WITH VARYING CONCENTRATIONS OF SERUM AC-GLOBULIN. These curves were obtained by adding varying amounts of serum Ac-globulin and a constant amount of prothrombin to thromboplastin and calcium ions under the conditions specified by the two-stage procedure for prothrombin analysis (20–22). At intervals, fibrinogen was added and clotting times were recorded. The amount of thrombin present is inversely proportional to clotting time (ordinate). The prothrombin and thromboplastin were relatively free of Ac-globulin as indicated by the slow formation of thrombin when no serum Ac-globulin was added. Under these conditions, one hour was required to produce enough thrombin to give 40-second clotting with fibrinogen. The prothrombin was of sufficient strength to give 12-second clotting with fibrinogen when fully activated. Concentrations of serum Ac-globulin (units per cc. x 1000) are represented by the large numbers at the tops of the curves and by the small numbers on the 24- and 18-second clotting time coordinates. The latter were derived by interpolation. The broken lines (rectangular curves) are for comparison, representing prothrombin activation curves obtained by the same method with two different concentrations of plasma Ac-globulin instead of serum Ac-globulin.

A unit of serum Ac-globulin is defined as 1000 times that amount which when present in one cc. of the reaction mixture will reproduce curve 1 of figure 3. This unit is approximately of the same magnitude as the arbitrary unit described for plasma Ac-globulin (18). One unit of plasma Ac-globulin should yield approximately one unit of serum Ac-globulin when the former is fully activated. It is unfortunate, however, that a precise correlation cannot be made with the use of data now available.

Stability of serum Ac-globulin. Serum Ac-globulin is remarkably stable in serum. Two samples of beef serum, collected by centrifugation one and one half hours after the blood was drawn, were stored at 5° C. The accelerator activity was compared at intervals with a pooled sample which had been frozen and stored at −30° C. The analytical values indicate, within the limits of experimental error, that no significant loss of activity occurred on storage at 5° C. for a period as long as 26 days (table 2).

TABLE 2. STABILITY OF ACCELERATOR IN BOVINE SERUM AT 5° C

DAYS OF STORAGE AT 5° C.	CONCENTRATION OF SERUM AC-GLOBULIN		
	Serum 1	Serum 2	Control serum[1]
	Units/cc.	*Units/cc*	*Units/cc.*
0	115	59	72
8	164	71	87
16	102	65	66
26	90	55	73

[1] Mixture of serum 1 and serum 2, stored at −30° C.

DISCUSSION

It has been mentioned repeatedly in the literature that in the clotting processes a 'latent period' precedes the period of thrombin production. After a small amount of thrombin is produced, further formation of thrombin apparently results from autocatalysis. The explanation for this phenomenon is furnished by our observations which have shown that Ac-globulin in plasma becomes more active in the presence of small amounts of thrombin (19, 24). Apparently the thrombin hastens its own formation by forming an intermediate (serum Ac-globulin) which, in turn, catalyzes further thrombin production. These reactions are more accurately termed co-autocatalysis (19, 24) instead of autocatalysis.

In order to attempt an explanation of the rôle of Ac-globulin in blood coagulation it is first necessary to mention that thrombin is produced slowly, in the absence of Ac-globulin, by the interaction of thromboplastin, prothrombin and calcium ions. This has been proven in this laboratory by extensive work which will be reported at a later date. This conclusion is in conflict with the recent interpretations presented by Owren (16).

From the prothrombin activation curves of figure 2, it is possible to conclude that plasma Ac-globulin itself does not act as a catalyst. It is a pro-enxyme and must first be changed, by thrombin, to the active catalyst, serum Ac-globulin. In figure 2, the curve at the extreme right represents the interaction of thromboplastin, prothrombin and calcium ions in the presence of plasma Ac-globulin. There was first a latent period, more accurately called a period of slow thrombin production, during which very small amounts of thrombin were formed by the interaction of thromboplastin, prothrombin and calcium ions. This period lasted for about 11 minutes during which time approximately 5 per cent of the prothrombin was converted to thrombin. The remaining 95 per cent of the potential thrombin was produced during the following two to three minutes. This period of rapid thrombin production results from the action of active catalyst and coincides with the formation of additional active catalyst. The same prothrombin, thromboplastin and calcium ions, alone forming thrombin at a slow rate, required from one to one and one half hours to produce a 30 to 40 per cent yield. When plasma was added which was previously activated with thrombin (10 units per cc.), the period of slow thrombin production was reduced to approximately two minutes. This acceleration of thrombin formation is much greater than was achieved with a comparable amount of untreated plasma. These facts make it very unlikely that active Ac-globulin exists in plasma; if there is any of the active catalyst in plasma, the amount is probably small as compared to the quantity of inert Ac-globulin present.

Assuming the above interpretations are correct, it is now possible to attempt a description of the processes of blood coagulation as they occur. The equation below represents the *first* reaction:

$$\text{Prothrombin} + \text{Thromboplastin} \xrightarrow{Ca^{++}} \text{Thrombin}$$

This permits the key concept that *serum Ac-globulin is the result of thrombin being formed first.* This reaction is slow but on the basis of mass law a high plasma prothrombin concentration presents favorable conditions. Perhaps that is why such an apparent excess of prothrombin is provided in plasma. The quantity of thromboplastin is the limiting factor in normal physiological conditions. The above reaction corresponds to the 'latent period' in blood coagulation. Even when 0.1 of a unit of thrombin per cc., about 0.03 per cent of the total potential thrombin of plasma, is liberated some serum Ac-globulin is produced (cf. table 1) as follows:

$$\text{Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Serum Ac-globulin}$$

This reaction is at first slow but as more catalyst (thrombin) becomes available it progresses more rapidly, producing more serum Ac-globulin. Meanwhile no clot has formed. However, the following reactions gain impetus:

$$\text{Prothrombin} + \text{Thromboplastin} \xrightarrow[\text{Serum Ac-globulin}]{Ca^{++}} \text{Thrombin}$$

$$\text{Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Serum Ac-globulin}$$

The thrombin titer soon rises high enough to cause clotting of fibrinogen as follows:

$$\text{Fibrinogen} \xrightarrow{\text{Thrombin}} \text{Fibrin}$$

Thereafter the above reactions become spent. The thrombin formed is destroyed by antithrombin and antithromboplastin acts on the thromboplastin (25–27). Serum Ac-globulin survives, or at least it does in the bovine species. Whether it does in other species remains to be determined.

It is theoretically possible to have serum Ac-globulin circulating in the living organism thus furnishing the individual with a hyperreactive coagulation system more sensitive to the action of thromboplastin than normal. An adequate source of thrombin, for forming the serum accelerator, could be derived from a bruise or from the vicinity of a thrombus. This may be of importance in the problem of thrombosis. It might be suggested that such minute amounts of thrombin would easily be disposed of by the action of plasma antithrombin. However, that is not in accord with the known facts of the reaction kinetics involved. It has been shown that the interaction of thrombin and antithrombin is an equilibrium reaction (28). The last traces of thrombin are removed with extreme difficulty (29) so that it is probably possible for small amounts of thrombin to remain active in the blood for enough time to partially activate plasma Ac-globulin.

It seems likely that serum Ac-globulin is identical with Factor VI of Owren (16). He concluded that Factor VI is formed from Factor V (plasma Ac-globulin?), but he did not recognize the key rôle of thrombin in this transformation. He, therefore, proposed that 'prothrombin? + Factor V $\xrightarrow{\text{Thrombokinase} + \text{Ca}^{++}}$ Factor VI' and that the latter is the true activator of prothrombin. The data reported above show that calcium ions are not necessary for the formation of the active accelerator, and they indicate that serum Ac-globulin is not the true activator of prothrombin. Owren did not recognize the basic difference between the plasma and serum type accelerators. In some experiments he used defibrinated plasma as a source of his Factor V products. He, therefore, used the plasma and serum accelerators interchangeably. It is also of interest that he reported a concentration of Factor V in serum as 190 per cent of that in plasma. This result is explained by the data reported above; serum Ac-globulin is capable of activating prothrombin much more rapidly than plasma Ac-globulin; a qualitative difference was mistaken for a quantitative difference in Owren's experiment.

SUMMARY

Plasma Ac-globulin is probably an inert protein or a pro-enzyme. It is changed to serum Ac-globulin by thrombin. Fibrinolysin will not produce the change. Serum Ac-globulin is the active catalyst of the interaction of prothrombin, thromboplastin and calcium ions. It is present in bovine serum and may be formed in oxalated plasma or from purified plasma Ac-globulin by the addition of small amounts of thrombin. Large quantities of thrombin destroy Ac-globulin activity. Thrombin is formed by co-autocatalysis in the blood-clotting mechanism.

Serum Ac-globulin has been obtained in concentrated form from bovine serum and from defibrinated oxalated bovine plasma. Its chemical properties cannot be differentiated from those of plasma Ac-globulin. Stability studies show that serum Ac-globulin is quite stable in bovine serum. Its activity can be measured quantitatively by the methods described.

The function of Ac-globulin in the blood-clotting mechanism is summarized by the following equations:

$$\text{(1) Prothrombin} + \text{Thromboplastin} \xrightarrow{\text{Ca}^{++}} \text{Thrombin}$$

$$\text{(2) Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Serum Ac-globulin}$$

$$\text{(3) Prothrombin} + \text{Thromboplastin} \xrightarrow[\text{Serum Ac-globulin}]{\text{Ca}^{++}} \text{Thrombin}$$

$$\text{(4) Fibrinogen} \xrightarrow{\text{Thrombin}} \text{Fibrin}$$

REFERENCES

1. SEEGERS, W. H., H. P. SMITH, E. D. WARNER AND K. M. BRINKHOUS. J. Biol. Chem. **123:** 751, 1938.
2. SEEGERS, W. H. J. Biol. Chem. **136:** 103, 1940.
3. SEEGERS, W. H. AND H. P. SMITH. J. Biol. Chem. **140:** 677, 1941.
4. SEEGERS, W. H., E. C. LOOMIS AND J. M. VANDENBELT. Arch. Biochem. **6:** 85, 1945.
5. SEEGERS, W. H., K. M. BRINKHOUS, H. P. SMITH AND E. D. WARNER. J. Biol. Chem. **126:** 91, 1938.
6. MERTZ, E. T., W. H. SEEGERS AND H. P. SMITH. Proc. Soc. Exp. Biol. and Med. **41:** 657, 1939.
7. SEEGERS, W. H. AND E. C. LOOMIS. Science **104:** 461, 1946.
8. SEEGERS, W. H. J. Physical and Colloid Chem. **51:** 198, 1947.
9. WARNER, E. D., K. M. BRINKHOUS AND H. P. SMITH. Proc. Soc. Exp. Biol. and Med. **40:** 197, 1939.
10. ZIFFREN, S. E., C. A. OWEN, G. R. HOFFMAN AND H. P. SMITH. Proc. Soc. Exp. Biol. and Med. **40:** 595, 1939.
11. OWEN, C. A., G. R. HOFFMAN, S. E. ZIFFREN AND H. P. SMITH. Proc. Soc. Exp. Biol. and Med. **41:** 181, 1939.
12. SMITH, H. P. Essays in Biology. Univ. Calif. Press, 1943. P. 549.
13. FANTL, P. AND M. NANCE. Nature **158:** 708, 1946.
14. WARE, A. G., M. M. GUEST AND W. H. SEEGERS. J. Biol. Chem. **169:** 231, 1947.
15. WARE, A. G., M. M. GUEST AND W. H. SEEGERS. Science **106:** 41, 1947.
16. OWREN, P. A. The coagulation of blood, investigations on a new clotting factor. J. Chr. Gundersen, Boktrykkeri, Oslo, 1947.
17. OWREN, P. A. Lancet **252:** 446, 1947.
18. WARE, A. G. AND W. H. SEEGERS. J. Biol. Chem. In press.
19. WARE, A. G., R. C. MURPHY AND W. H. SEEGERS. Science **106:** 618, 1947.
20. WARNER, E. D., K. M. BRINKHOUS AND H. P. SMITH. Arch. Path. **18:** 587, 1934.
21. WARNER, E. D., K. M. BRINKHOUS AND H. P. SMITH. This Journal **114:** 667, 1936.
22. SMITH, H. P., E. D. WARNER AND K. M. BRINKHOUS. J. Exp. Med. **66:** 801, 1937.
23. SEEGERS, W. H. AND MCGINTY, D. A. J. Biol. Chem. **146:** 511, 1942.
24. SEEGERS, W. H. AND A. G. WARE. Proceedings of the Canadian Physiological Society, 1947. P. 29.
25. TOCANTINS, L. M. This Journal **139:** 265, 1943.
26. TOCANTINS, L. M. Proc. Soc. Exp. Biol. and Med. **57:** 211, 1944.
27. SCHNEIDER, C. L. This Journal **149:** 123, 1947.
28. SEEGERS, W. H., E. D. WARNER, K. M. BRINKHOUS AND H. P. SMITH. Science **96:** 300, 1942.
29. SEEGERS, W. H. AND H. P. SMITH. Proc. Soc. Exp. Biol. and Med. **52:** 159, 1943.

INFLUENCE OF HEPARIN ON THE OPTICAL DENSITY OF CLOTTED PLASMA[1]

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In assaying heparin by the use of beef and sheep plasma, the problem of assessing the end-point led to a study of the optical density of the clot. The transmission of light as a means of studying coagulation of plasma has been used by various workers (1). The clotting of fibrin in a system of purified components was reported by Ferry and Morrison (2). These studies did not include heparin. Meylink (3) proposed the use of the electrophotometer for assaying heparin but gave no data and stated that the end-point should be taken at corresponding points on the coagulation curves in the region where conversion of fibrinogen is most rapid.

The present investigation was undertaken with the same object in view as Meylink's study, that is, to determine the applicability of the optical method to the assay, and was in progress when Meylink's report became known. An assay could be carried out in two ways: 1) establish time-density clotting curves for different concentrations of each sample and standard and make proper comparisons, or 2) set up the experiment as in the usual assay technique and after one or two hours read the degree of clotting with the electrophotometer instead of by the visual method. The first method is not adaptable for practical assay work because of the limited number of samples that can be run simultaneously, and because the rapid deterioration of plasma obviates quantitative comparison of samples not run concurrently. The chief objection to the second method (and it applies to the first as well) is the lack of uniformity of the clots in many instances, more particularly in beef than in sheep plasma (4). This lack of uniformity can lead to considerable error in determining the light transmission since in practically all photoelectric instruments the light beam passes through only a portion of the medium.

This report covers investigations on time-density clotting curves as determined by the photoelectric method. It is not concerned with assay material as proposed under 2) above and it will be seen that the use of the clotting curves themselves cannot be adapted very well to an assay method. A preliminary report on this study was given at the 1947 Federation meeting (5).

METHOD

Optical density has been expressed in terms of reciprocal function, the per cent transmission of light. Measurements were made with a Fisher electrophotom-

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[1] Supported by a grant from the United States Public Health Service.

eter. Thirteen x 100 mm. lipless pyrex tubes selected for uniformity were employed. The tubes with cork stoppers were too tall for the cover of the instrument to close tightly so a section of it was cut out and a light tight hood attached.

The procedure consisted of recalcifying citrated plasma in the presence of varying concentrations of heparin. This was similar to the assay technique (4). Beef and sheep plasma stored in the frozen state were used. The volume was 3 cc. of which 1.5 cc. represented the plasma. A volume of 2 cc., employed in the assay, could not be used on account of the size of the light aperture, and it seemed better to increase the volume rather than alter the instrument. The ingredients were added in the following order: heparin, plasma, saline and calcium chloride. After adding calcium the tubes were immediately stoppered with paraffined corks, inverted four times and placed in the instrument. Time was measured from the moment of inversion. The tests were conducted at room temperature. The red filter was used in the electrophotometer.

The first or 'zero' reading was made at one minute and subsequent readings at appropriate intervals thereafter. Two tubes were run simultaneously. The instrument was first adjusted to read 100 per cent transmission using distilled water as the standard. Plasmas then showed approximately 95 per cent transmission.

Curves of the results were drawn by plotting the per cent transmission against the logarithm of the time in minutes. Quantitative comparisons are valid only between two tubes run simultaneously. Because of gradual deterioration of the thawed plasma all comparisons between tubes run at different times must be considered qualitative.

Species and individual variations of plasma (fig. 1). Coagulation in unheparinized plasma was followed for several hours. The amount of light transmitted was reduced to about 65 per cent in sheep plasma and about 20 per cent in beef plasma. Curves for two samples of each species are shown in figure 1. One sample of each species was a composite of several batches mixed on the day of collection. The curves illustrate the marked species difference in clot density and the variation between specimens to be expected. These curves represent approximately the extremes encountered among the plasmas tested.

It may be noted also that the time of onset of clotting varies. One curve of each species shows a delay of five minutes. Both of these samples were obtained in winter. The composite samples were obtained in summer and though the inception of clotting was more rapid the final densities did not reflect the same apparent seasonal effect. Among data so far accumulated the optical density has usually been greater in plasmas collected in summer than in those collected in winter. However, in figure 1 the winter sample of sheep plasma (SE) is denser. Additional tests are needed to distinguish clearly between seasonal and individual variation. It may be added that in assay work there is a tendency for both species to require less heparin in winter than in summer thus reflecting seasonal density changes. A similar observation was made by Jorpes and his coworkers (6).

Nygaard (7) noted that the plasma clotting curve is divided into four parts.

The first phase is termed the period of dissociation. It corresponds to stage 1 of the clotting reaction and is the period from mixing to the beginning of fibrin formation, a point designated *F* by him (see fig. 1). At *F* the transmission of light begins to decrease rapidly. The second phase is the period of fibrin formation and is represented by the rapid descent of the curve. Fibrin formation is considered almost complete at a point *C* located where the curve begins to level off. Beyond *C* is the third phase or 'rest period'. This 'rest period' is characterized

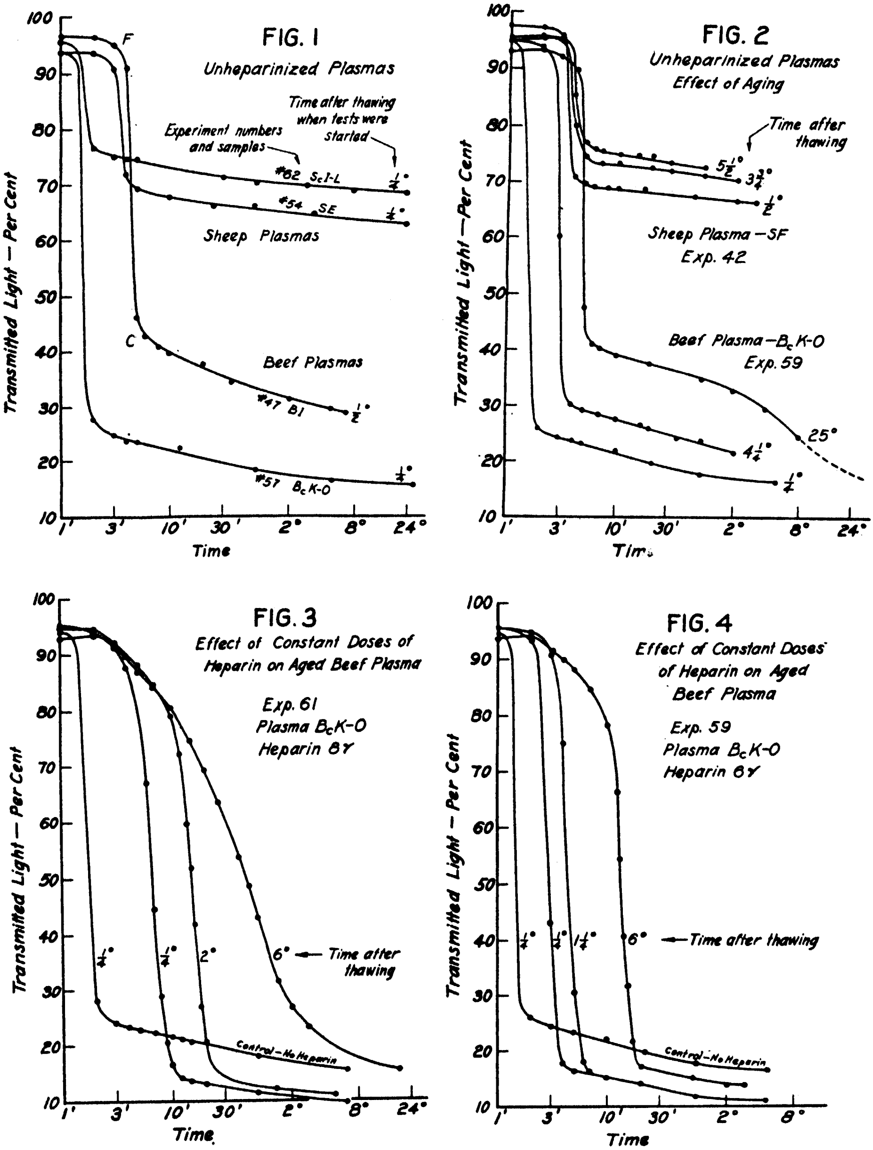

FIG. 1-4

by a further gradual decline in light transmission (or increase in density). This decline is quite apparent in the curves shown here and, in fact, proceeds much farther than in Nygaard's curves, probably attributable to the absence of clot retraction in these tests in contrast to his tests on fresh human plasma. The fourth phase is the phase of retraction, the onset designated *R* by Nygaard, and is shown by him to start within a few minutes after point *C* is reached. Retraction has never been observed in clots from frozen beef or sheep plasma, presumably because of loss of platelets. The glass surface may be a factor in preventing retraction of the colloidal aggregate (8) but retraction has not been seen in cellulose nitrate or silicone coated glass tubes.

In the third phase or 'rest period' Nygaard first considered that the further increase in density might be due to clot retraction without expression of serum. He ruled this out however by showing that further fibrin was formed during this third phase. The apparent length of this third phase was short in his experiments. If the same explanation holds for these tests, it means that coagulation is not complete for at least 8 to 24 hours. The plasmas employed here, in most instances, began to coagulate as soon or sooner than in the examples shown by Nygaard and the second phase was just as rapid. It does not seem unlikely that additional fibrin might be formed during the early part of the 'rest period', but it hardly seems credible that fibrin may still be forming for as long as 8 to 24 hours. However, the curves show a decrease in light transmission during the 'rest period' of as much as 30 per cent with or without heparin and we would then infer that 30 per cent more fibrin is formed during this period. Since the changes between the second and third phases are somewhat abrupt, this would suggest that the transformation of fibrinogen is substantially completed by this time.

It seems more plausible that some physical transformation of the fibrin already formed is taking place. Retraction—or contraction—of individual isolated fibrils may be taking place, thereby becoming shorter and thicker and with an absence of fusion to form an interlacing network would then result in no retraction of the clot as a whole. Another explanation may be that the fibers are simply imbibing fluid and swelling. Finally, a third explanation may be that the fibrils are agglutinating to form larger fibers but are not contracting. In any event a thickening of the fibrin fibers would account for the greater optical density. One of these explanations seems more likely than that fibrin formation is still going on for several hours and to the extent of 30 per cent.

Some of the terms used by Nygaard (7) do not seem appropriate. His designation of the first phase as 'dissociation' implies that the first stage of the clotting reaction is a dissociation process but, at the most, this is only the initial part of the reaction since molecules are also considered to be combining during this phase. It would seem more simplified and less committal to call the first phase the 'latent period' on these clotting curves. The second phase, that of fibrin formation, could be described as the 'coagulation period'. The third phase, or 'rest period', is certainly not characterized by rest. The term 'condensation' used by Howell (8) is more appropriate although he considered this condensation to be a part of the process of retraction. But it is manifest that condensation can occur without clot retraction. The fourth phase, or 'retraction period', is suitably named. In this paper the first three phases will be termed the latent, coagulation and condensation periods, respectively.

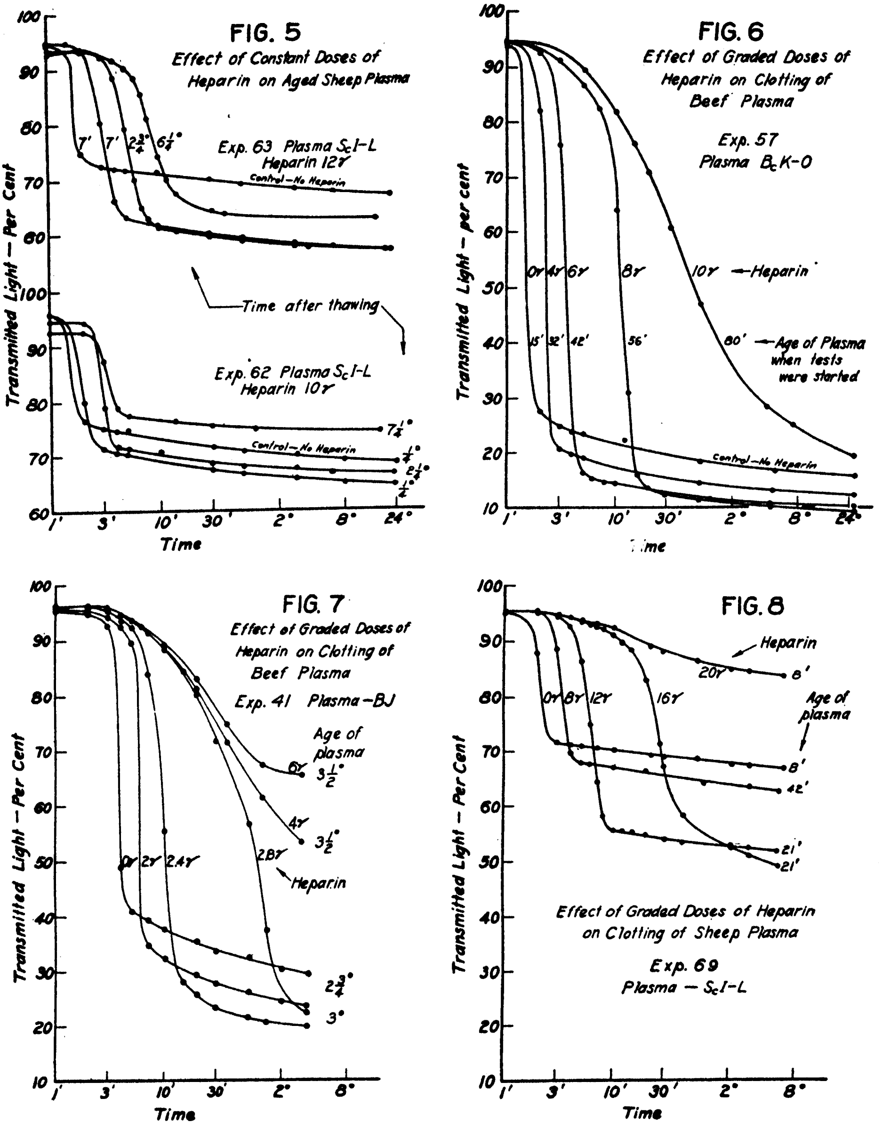

FIG. 5–8

Effect of aging of thawed plasma (fig. 2). It has long been known that plasma loses it coagulability on standing for sufficient time at room temperature. The curves in figure 2 show both a decreased final density and a delay in the onset of clotting which are evidence of this loss. The effect is more noticeable with beef than with sheep plasma. The decreased density may be explained by a loss or denaturing of the fibrinogen. Lavergne *et al.* and Lozner *et al.* (9) have given evidence for the loss of fibrinogen in aged plasma. However, Honorato and Quick (10) do not believe that fibrinogen is lost and Honorato (11) states that fibrinogen from stored plasma clots more easily.

Effect of heparin on aged thawed plasma (*fig. 3, 4 and 5*). A constant concentration of heparin was used in each series of tests. Two different concentrations are shown here for both the beef and the sheep plasmas. The onset of the clotting was delayed in all instances and the delay became progressively greater as the plasma aged. The latent period is thus a summation of the aging effect and the inhibition caused by heparin (cf. fig. 2).

In all tests where heparinized and unheparinized plasma were tested simultaneously, the final density of clots formed in the presence of heparin became greater. As the plasma aged the final clot density in the presence of heparin became less but in most cases was still greater than that of the unaged control plasma. Experiment 62, figure 5, shows a case with sheep plasma where aging at last resulted in a final density less than that of the original plasma.

Effect of graded concentrations of heparin (*fig. 6, 7 and 8*). The tests in experiments 57 and 69, figures 6 and 8, were made as soon after thawing as possible. Since only two tests could be run simultaneously, varying lengths of time necessarily elapsed before the entire range could be covered. The aging factor has but a small influence on these results as may be concluded by comparing the curves with those of figures 2 to 5 and may be neglected in the interpretations. Nevertheless, the actual plasma ages at the time the tests were started have been indicated on the charts.

Figure 6 shows the reaction of a composite beef plasma to concentrations of heparin ranging from 4 to 10 gammas per tube (that is, per 1.5 cc. of plasma). The latent period is progressively prolonged and the maximum rate of clotting has become diminished. However, when the effect is plotted against the log of time, the steepest portions of the curves are seen to be nearly parallel with all but the highest concentration of heparin. The curves show that there is a progressive increase in the final clot density as the dose of heparin is increased until a concentration between 8 and 10 gammas is reached.

Figure 7 shows curves of a sample of beef plasma tested after it had aged about three hours. The results were the same as those in figure 6 except for the much lower range of heparin concentration. The markedly different heparin requirement is attributed to plasma variation (the plasma was collected in winter).

In figure 8 results on sheep plasma are given. The curves are quite comparable to those for beef plasma, for as the dose of heparin is increased the ultimate density of the clot increases until a critical concentration is reached where the plasma rapidly fails to clot. In the coagulation period the curves are all parallel in their steepest portions except for the one with the highest dose of heparin. The increased final density was particularly marked with 12 and 16 gammas of heparin, the proportionate increase being greater than with beef plasma and even greater than usually encountered with sheep plasma. The plasma was a summer specimen.

DISCUSSION

It is now apparent that an assay technique for heparin in which density-time clotting curves were to be established would not be practical. The chief reason for the lack of adaptability is the change in the coagulation characteristics as the plasma ages after thawing. As a means of reading the end-point in an assay by conventional techniques, the electrophotometer may be of use. At least the end-point would be purely objective and not subject to the personal errors of visual observation in the interpretation of the degree of clotting. Since the dose-effect curve is very steep (4), it is questionable whether in practical assay work the use of the electrophotometer would add significantly to the accuracy of the result. A study of this is planned however.

These studies have clearly shown the effect of heparin and aging of the plasma in modifying the character of the time-density clotting curve. Aging results in a decrease in the density of the clot and heparin within limits increases its density. Both aging and heparin tend to prolong the latent period. The rate of coagulation is decreased by heparin, but the charts show that the steepest portions of the curves are approximately parallel so the rate of fibrin formation is a constant function of the log of time up to the point where the plasma rapidly becomes incoagulable.

The explanation for effect of heparin, in increasing the density of the ultimate clot, must lie in the rate of formation of fibrin. Slow formation of the fibrin fibers results in growth to a larger size and is analogous to the precipitation and growth of crystals from solutions of crystalloidal substances. Rapid formation of particles, whether crystalloidal or colloidal in nature, results in their being smaller and more highly dispersed. Heparin controls the concentration of one of the two factors of the second stage of the clotting reaction. By keeping thrombin at a low concentration, the conversion of fibrinogen molecules is induced to proceed more slowly and to go to a degree of greater particle size and, hence, greater optical density. Howell (12) observed in the ultramicroscope that rapid clotting gave fine needles of fibrin and that slow clotting gave large needles. Wolpers and Ruska (13) in studies with the electron microscope employed heparin to slow the rate of clotting and allow the growth of larger fibers. Indirect evidence has been obtained in, as yet, incomplete experiments for attributing the density to increased particle size. Clots were subjected to high speed centrifugation (about 18,000 G), dried and weighed. In the heparin concentration range, where optical density was increasing, the clots became lighter in weight.

The question of the gradual increase in optical density of the ultimate clot during the condensation period has already been discussed.

SUMMARY

1. The changes in optical density taking place in the clotting of plasma were followed with an electrophotometer. The plasma employed had been kept frozen for several months.

2. The optical density of clotted beef plasma is about three times as great as that of clotted sheep plasma.

3. As the plasmas age after thawing there is a delay in the onset of coagulation (latent period), a slightly slower rate of coagulation and a final clot of diminished density. After the phase of rapid coagulation (coagulation period) is completed the optical density of the clots continues to increase (condensation period), probably reflecting a physical change in the fibrin.

4. Heparin in all doses prolongs the latent period.

5. With the lower concentrations of heparin the coagulation period is prolonged but the logarithm of the rate of fibrin formation is constant.

6. With the lower concentrations of heparin the final optical density of the clot becomes progressively greater as the concentration increases. With the higher concentrations of heparin the plasma becomes rapidly incoagulable.

7. The optical density continues to increase for many hours during the condensation period.

REFERENCES

1. Klinke, K. and G. Elias. Z. ges. exper. Med. **77**: 717, 1931.
   Wohlisch, E. and W. Krapf. Pflueger's Arch. **238**: 41, 1936.
   Baldes, E. J. and K. K. Nygaard. Proc. Staff Meet. Mayo Clin. **11**: 151, 1936.
   Feston, H. Munch. med. Wschr., **84**: 1370, 1937.
   Ebbecke, U. and F. Knuchel. Ibid. **243**: 54, 1939.
   Deutsch, H. F. J. Clin. Invest. **25**: 37, 1946.
2. Ferry, J. D. and P. R. Morrison. J. Am. Chem. Soc. **60**: 388, 1947.
3. Meylink, B. Ned. Tijdsch. v. Geneesk. **90**: 24, 1936.
4. Foster, R. H. K. J. Am. Pharm. Assoc. **36**: 244, 1947.
5. Foster, R. H. K. and A. J. Begany. Federation Proc. **6**: 330, 1947.
6. Jalling, Q., J. E. Jorpes, and G. Linden. Quart. J. Pharm. Pharmacol. **19**: 96, 1936.
7. Nygaard, K. K. Hemorrhagic Diseases: Photo-Electric Study of Blood Coagulability. St. Louis, C. V. Mosby, 1941. P. 68.
8. Howell, W. H. This Journal **40**: 526, 1916.
9. Lavergne, G. H. and B. Lavergne-Poindessault. Compt. rend. Soc. de biol. **136**: 445, 1945.
   Lozner, E. L., F. H. L. Taylor, S. Lemish, R. Snyder and L. R. Neuhouser. J. Clin. Invest. **23**: 357, 1944.
10. Honorato, R. and A. J. Quick. This Journal **150**: 405, 1947.
11. Honorato, R. This Journal **150**: 381, 1947.
12. Howell, W. H. This Journal **35**: 143, 1914.
13. Wolpers, C. and H. Ruska. Klin. Woch. **18**: 1077 and 1111, 1939.

IMPORTANCE OF PRESSURE FACTORS IN THE GENESIS OF PULMONARY EDEMA FOLLOWING VAGOTOMY[1]

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Bilateral cervical vagotomy results, in several species of animal, in pulmonary edema. Various workers, recently Lorber (1), have shown that obstruction to the airway by laryngeal paralysis and accumulation of tracheal secretions plays a large part in the pathogenesis of edema following vagotomy. They found that if a free airway is maintained lung edema does not occur. On the other hand, Farber (2) has reported the results of experiments in which guinea pigs were maintained on artificial positive pressure respiration after tracheal cannulation. He found in such animals that fatal lung edema occurred in three and one-half to four hours if bilateral cervical vagotomy had been performed, while animals with intact vagi showed only congestion after five hours. He interpreted his findings to mean that "disturbances to the vasomotor control of the pulmonary vessels . . . lead to the production of severe pulmonary edema and hemorrhage."

Since the studies of Daly (3) and others show only slight evidences of pulmonary vasomotor activity in vagal fibers it seems unlikely that the above conclusion is correct. For this and other reasons it was decided to study further the factors involved in lung edema following vagotomy in the guinea pig. As a first step the experiments of Farber were repeated. This paper is a report on the observations made.

METHODS

Adult guinea pigs of both sexes were employed. They were anesthetized with intraperitoneal nembutal, 3.0 mgm. per 100 grams. Artificial respiration by positive pressure tracheal cannula insufflation was maintained throughout. Two animals, one with cervical vagotomy and one a control, were studied simultaneously on the same respiration pump. The pressure of insufflation was varied in different experiments. Expiration was passive, at atmospheric pressure. All animals were killed by bleeding from both cut carotids. To obtain constancy of conditions for study of the lungs the lungs were not removed until bleeding had ceased with the hind legs elevated. Because no accurate chemical method of assay of pulmonary edema has been developed the state of the lungs was assessed by gross and microscopic examination. The following index was used to grade the gross lesions:

Grade 1. Slight deepening of the normal pink.

Grade 2. Moderate generalized red discoloration.

Grade 3. Deep red congestion of the lungs but no frothy fluid in the trachea or dripping from the lung surface.

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[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

Grade 4. Frothy serosanguinous fluid in the trachea, bloody fluid dripping from the lung surface but considerable normal lung remaining.

Grade 5. Bilateral complete involvement with extremely wet deeply hemorrhagic lungs.

TABLE 1

INTACT VAGI GROUP					VAGOTOMIZED GROUP				
Weight	Sex	Duration Hours		Index of Lung Pathology	Weight	Sex	Duration Hours		Index of Lung Pathology
322	M	4	KO	1	250	M	7	KO	2
260	M	5	KO	1	240	M	11	KO	3
350	M	5	KO	2	245	F	7½	KO	2
270	M	5	KO	1	366	M	9	KO	3
265	F	7½	KO	3	520	F	9½	KO	3
510	M	10	KO	1	850	M	9½	KO	2
530	F	5	K	1	510	F	9	KO	2
407	M	5	K	1	611	M	5	K	1
720	M	12	K	1	650	M	12	K	2
668	F	14	K	1	1000	M	14	K	2
794	M	25	K	3	774	M	25	K	3
Mean Index—1.5					Mean Index—2.2				

K—Killed.
O—Open chest experiment.

TABLE 2

Respirator pressure 20 mm. Hg

INTACT VAGI GROUP					VAGOTOMIZED GROUP				
Weight	Sex	Duration Hours		Index of Lung Pathology	Weight	Sex	Duration Hours		Index of Lung Pathology
354	M	3½	K	4	367	M	3	K	4
460	M	5	K	5	456	M	5	K	4
575	F	5	K	5	459	M	5	K	4
515	M	6	K	5	852	M	6	K	4
Mean Index—4.7					Mean Index—4				

K—Killed.

RESULTS

In Table 1 appear the results of 11 pairs of experiments in which guinea pigs were maintained on 6 mm. Hg positive pressure insufflation for periods ranging from 4 to 25 hours. It may be noted that in no instance was pulmonary edema observed. The mean index of lung pathology was slightly higher in the vagotomized than in the control group, but the former were maintained longer on artificial respiration before sacrifice in 6 of the 11 cases. The important point seems to be that congestion was the maximum lesion seen, even in vagotomized animals, after as much as 25 hours of artificial respiration at 6 mm. Hg pressure.

On the other hand, as may be seen in table 2, when 20 mm. Hg pressure was employed in the artificial respiration an entirely different picture is found. In four pairs of experiments every animal showed massive edema within from 3 to 6 hours. In this series it may be noted that the mean index of lung pathology is lower in the vagotomized group.

It will be noted that in certain experiments in table 1 it is indicated that the chest was open. No remarkable difference was seen as a result of this maneuver. Microscopic studies were made on representative samples of lung tissues from these experiments.[2] The results will not be reported in detail because they merely confirm the gross observations in the case of the more severe two grades of pathology. The less severe changes seen grossly cannot be accurately distinguished in microscopic section.

DISCUSSION

These studies show that pulmonary edema does not occur either in the intact or the vagotomized guinea pig under artificial respiration, when the pressure used is 6 mm. Hg within periods of from 5 to 25 hours. Such edema does occur, however, within 5 hours in both intact and vagotomized animals when the pressure of insufflation is 20 mm. Hg. The earlier studies cited reporting such edema probably dealt with pressures in the latter range, although no mention was made of pressure conditions in the report. The present studies do not account for the reported differences between intact and vagotomized guinea pigs. A systematic difference in experimental and control animals could if it occurred, account for Farber's results. It is also possible that different results might be obtained at pressures intermediate between the two employed in the present study.

The present results seem to show quite clearly that pressure factors are critical for pulmonary edema formation in the guinea pig under artificial respiration. Furthermore, they show that under at least one pressure condition under which lung edema occurs regularly, it is not augmented by vagotomy. It seems very doubtful that vagotomy exerts any important specific effect upon the susceptibility of the lung vessels to edema or hemorrhage, although secondary effects mediated through known vagal actions on the heart, the bore of the bronchial tree, or on the reflex control of respiration, are not impossible nor even unlikely.

The observations reported at this time appear to be significant also in showing that in the guinea pig, as in the dog (4), positive pressure respiration does not prevent the development of lung edema under certain conditions. It should not, however, be concluded that under other conditions positive pressure may not impede edema formation. It is not impossible that extreme, prolonged positive pressure breathing may induce such a degree of cardiac failure as to raise left atrial and pulmonary venous pressures to very high values, thus inducing pulmonary edema. This possibility is at present being tested.

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[2] The authors are indebted to Mr. James Weiss for preparation of the microscopic sections.

SUMMARY

1. Neither intact nor vagotomized guinea pigs developed pulmonary edema when maintained on artificial respiration at 6 mm. Hg insufflation pressure. Both intact and vagotomized animals developed massive edema within 6 hours when the insufflation pressure was raised to 20 mm. Hg.

2. Under the conditions of these experiments there is no evidence that vagotomy exerts an influence on the pulmonary vascular system favoring edema or hemorrhage.

REFERENCES

1. Lorber, V. J. Exp. Med. **70**: 117–30, 1939.
2. Farber, S. J. Exp. Med. **66**: 405–11, 1937.
   Farber, S. Arch. Path. **30**: 180–197, 1940.
3. Daly, I. de B., S. R. Elsden, C. O. Hebb, G. V. Ludany and Barbara Petrovskaia. Quart. J. Exp. Physiol. **31**: 227–262 (1941–42).
   Daly, I. de B. and Catherine O. Hebb. Quart. J. Exp. Physiol. **31**: 211–226 (1942).
4. Zinberg, S., G. Nudell, W. G. Kubicek and M. B. Visscher. Amer. Heart J. In press.

AN ATTEMPT TO PRODUCE PULMONARY EDEMA BY INCREASED INTRACRANIAL PRESSURE[1]

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Several reports have indicated that the central and antonomic nervous systems may be implicated in the production of some types of paroxysmal pulmonary edema (1–6). Thus Luisada (1) has claimed that in rabbits pulmonary edema of a reflex nature may be produced experimentally by inducing hypertension in the isolated head. Further, cases of paroxysmal pulmonary edema have been reported to have occurred following cranial injury (7). Our interest in the mechanism of the blood pressure changes occurring during increased intracranial pressure (8) made it appear of value to study the mechanism of the pulmonary edema which reportedly occurred under these conditions. For this purpose, we employed increased intracranial pressure which, while directly affecting the higher nervous centers, at the same time causes an increased load upon the left ventricle due to resulting elevated systemic arterial pressure.

METHODS

Dogs were anesthetized with pentobarbital sodium (25 mgm./kgm.). Arterial and intracranial pressures were measured with mercury manometers. Intracranial pressure was raised by means of a saline reservoir connected through a trephine hole in the skull, the underlying dura mater having been removed. We attempted to maintain the intracranial pressure at such a level that the resulting arterial pressure rise would be maximal, without producing acute failure of the left ventricle. Under these circumstances the factor limiting survival of the animal was the failure of respiration which always occurred before dynamic impairment of cardiac function was detected. Because of this tendency of increased intracranial pressure to cause respiratory arrest, the intracranial pressure was applied intermittently and its level was varied with the condition of the animal. Respiration was spontaneous (except as noted below), positive blast artificial respiration being avoided as an unfavorable factor for the production of pulmonary edema.

RESULTS

In two of our early experiments in which increased intracranial pressures of from 20 to 138 mm. of Hg had been maintained intermittently for periods totalling about 71 minutes, the lungs were full of a copious, thin, white, frothy fluid present in the air passages as far down as the dissecting scissors could reach and expressible from the cut surface of the lung. The picture grossly was that of massive pulmonary edema. However, we noted that after application of the increased intracranial pressure, many of the animals tended to produce considerable amounts of saliva, and the possibility that this saliva might be a contributing factor in the production of this picture had to be excluded. Accordingly, the experiments were repeated in nine dogs in which tracheotomy was performed so as to prevent saliva from passing down the trachea into the lungs. Some of the animals were maintained during the experiment with the head slightly dependent. Two of the animals in this group had the chest opened and received positive blast respiration which permitted the application of intracranial pressures up to levels (over 300 mm. Hg) above that which the arterial pressure could attain. One other animal received positive blast respiration with the chest closed. The other six animals breathed naturally. In none of these animals was there any evidence of pulmonary edema.

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[1] Aided by the A. D. Nast Fund for Cardiovascular Research. The Department is supported in part by the Michael Reese Research Foundation.

Microscopic examination of the lungs of the two original dogs without tracheal cannulae, which had showed fluid in the air passages, revealed bronchopneumonia in one dog and anthracosis in the other. No true pulmonary edema was present in either case.

DISCUSSION

Short (9) has already pointed out that the aspiration of stomach contents and the accumulation of tracheal and bronchial secretions leading to slow asphyxia may result in the development of pulmonary edema. It is apparent that the increased intracranial pressure as produced in our experiments did not produce pulmonary edema. However, our experience serves to show that a picture grossly resembling pulmonary edema may be produced by the aspiration of saliva. Our experience serves to question whether previous attempts to produce pulmonary edema by increasing intracranial pressure were successful or were due to artefact resulting from the aspiration of saliva.

REFERENCES

1. Luisada, A. Boll. d. Soc. ital. di biol. sper. **5:** 528, 1930.
2. Fontaine, R. and Courtine, G. Presse. Med. **48:** 711, 1940.
3. Weber, F. P. and Blum, K. J. Neurol., Neurosurg. and Psychiat. **5:** 37, 1942.
4. Bykov, K. M., Potapova, V. M. and Rikkl, A. V. Byul. eksper. biol., i. med. **15:** 40 1943.
5. Reichsman, F. Am. Heart J. **31:** 590, 1946.
6. Luisada, A. A. and Sarnoff, J. J. Am. Heart J. **31:** 270, 282, 293, 1946.
7. Schlesinger, B. J. Nerv. & Mental Dis. **102:** 247, 1945.
8. Rodbard, S., A. Surtshin, and L. N. Katz Unpublished observations.
9. Short, R. H. D. J. Path. & Bact. **56:** 355, 1944.

RETENTION OF SEX FUNCTIONS AFTER ISOLATION OF THE PARS ANTERIOR BY EXTIRPATION OF THE HYPOPHYSIAL STALK[1]

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A long-term cytological study of the canine hypophysis is being made with special attention directed to the tissue remaining after graded hypophysectomy procedures (1, 2). It is suspected that such an approach, when correlated with the careful assaying of the functional deficits produced in the animals, will yield information relative to the association of function with specific anatomical elements that perhaps has not hitherto been attained.

The present report summarizes the findings with respect to sex functions in a group of nine dogs in which the hypophysial stalk tissue was surgically removed with a sparing of varying amounts of the distal portion of the pars anterior as illustrated in the diagram and photograph shown in figure 1.

MATERIALS AND METHODS

Animals. There were one male and eight female mongrel dogs of medium size used in this series. At the time of operation five animals were sexually mature, while the others were 'senior' pups. The animals were allowed to exist for periods of time ranging from 8 to 19 months.

Operative procedure. After the right lateral aspects of the hypophysis, the stalk, and the hypothalamus were exposed by the subtemporal approach, as previously described (1), the stalk tissue was removed in one of the following two ways.

In the first part of the series the stalk was cut distally with one snip of the scissors in such a manner that an appreciable rim of pars anterior tissue remained attached to the stalk tissue (see line 2, figure 1A). A small metal disc was then placed against the cut surface of the isolated hypophysial tissue to protect it from the heat of the cauterization probe. The stalk tissue which remained attached to the hypothalamus was then cauterized.

This method was soon discarded because of 1) the uncertainty of devitalizing all the stalk tissue and 2) the frequent encroachment upon the tuberal portion of the hypothalamus. Instead, as an alternative procedure, the stalk was cut with the scissors as close to the hypothalamus as possible (see line 1, figure 1A), and a second cut was made through the proximal portion of the pars anterior (see line 2, figure 1A). The tissue freed by the two incisions was removed as a surgical specimen and stored in formalin for reference. This procedure prevented encroachment upon the hypothalamus, as well as leaving no more than a remnant of the proximal stalk attached to it.

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[1] Aided by a grant from the John and Mary R. Markle Foundation.

Tissue. At autopsy the brain was removed from the floor of the skull and sella turcica in such a manner that the meninges, connective tissue, and hypophysial fragments remained in situ on its under surface.

Following fixation of the hypophysio-hypothalamic block in Zenkerformol and a subsequent period in 10 per cent formalin, the tissues were sectioned at 6 micra. An interrupted series (one section from each 20) was stained by the Mallory azan method. Another series was subjected to the copper chrome hematoxylin of Rasmussen (3). The latter stain is considered as specific for the acidophilic cells of the anterior lobe. Hansen's chromalum hematoxylin, as mentioned by Dawson (4), was used as a selective stain for the basophilic cells, but was not used for the whole series, since it was considered to have no distinct advantage over the Mallory azan method.

RESULTS

Accomplishment of operative objective. The hypophysial stalk tissue, with the exception of small fragments of proximal tuberalis and infundibulum which remained attached to the hypothalamus, was removed in all instances. This desired effect was accomplished at the expense of considerable encroachment upon the proximal pars anterior tissue and resulted in varying amounts of the distal pars anterior remaining viable. There was slight infringement upon the hypothalamus in two of the dogs, while in the others microscopic inspection revealed the hypothalamus to be undisturbed. A photomicrograph of a section taken from the series on *dog 1* is shown in figure 1B.

Retention of sex function. Completely normal sex functions were retained in *dogs 1* (female) and *2* (male), as shown by the female's whelping and rearing a litter of five puppies which were sired by the male. Sex functions were also retained in *dogs 3*, *4*, *5*, and *6*, as judged by the fact that autopsy revealed genital tracts of normal size and serial sections of the ovaries demonstrated that the numbers and structure of the follicles were within normal limits. Regressing corpora lutea were observed in these sections in *dogs 3*, *4*, *5*, and *6*.

Dog 1 was sexually mature at the time of operation. The first postoperative estrus period occurred in the fifth month during which she copulated with a normal male several times, and nine weeks later whelped one pup. Estrus was again manifested six months later, but she was not mated. In the third estrus period, which occurred six months later, she was mated with *dog 2* and whelped a litter of five pups.

Approximately the distal half of the pars anterior was found to have remained viable when the animal was terminated 19 months after the operation. This is shown in the photograph in figure 1B. Histologically the sections showed a normal complement of acidophiles, basophiles and chromophobe cells. The chromophilic cells were well granulated, and the arrangement of the cells in the structure of the cords did not deviate markedly from the normal. These features are illustrated in the photomicrograph shown in figure 2A.

Dog 2 was judged to be approximately three-fourths grown and was sexually immature at the time of operation. Subsequently he matured, without any suspicion of delay, and exhibited a *persistent hyperactive sex drive*. At autopsy 16 months after operation, approximately the distal half of the pars anterior remained intact and acidophiles, basophiles and chromophobe cells were well represented in the glandular tissue. The only deviation from a normal appearance was that the arrangement of the cells tended more to a diffuse pattern with slightly enlarged sinusoids intervening between the cords of cells.

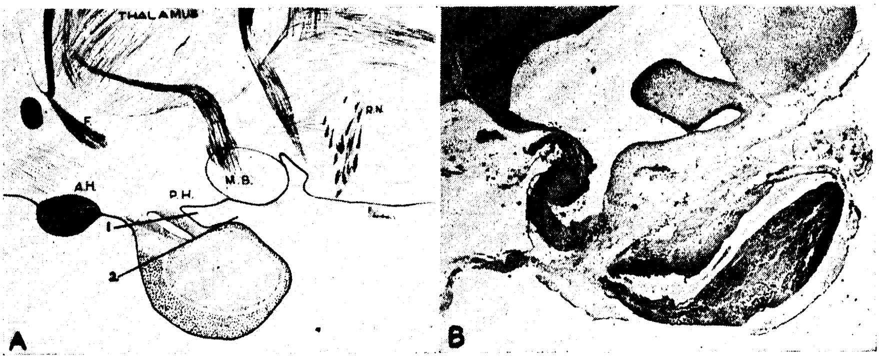


FIG. 1A. DIAGRAMMATIC REPRESENTATION OF A SAGITTAL SECTION through the hypophysis and hypothalamus to illustrate the levels of section and the amount of tissue removed as a surgical specimen.

FIG. 1B. LOW POWER PHOTOMICROGRAPH of a section of the hypophysis and hypothalamus taken from the series on *dog 1*. Optic chiasma is located to upper left of figure. Note that a fibrous layer is interposed between the infundibulum that remains and the distal hypophysial tissue. The cleft which separates the pars anterior from the pars intermedia and the remnant of the pars nervosa is clearly evident. Note particularly that no pars tuberalis tissue remains.

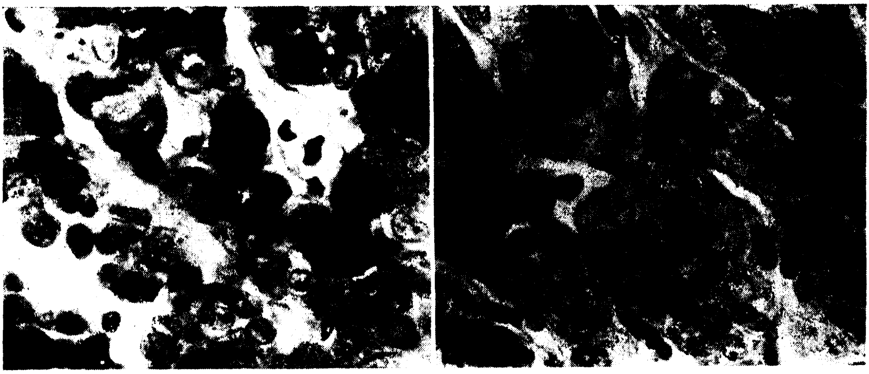
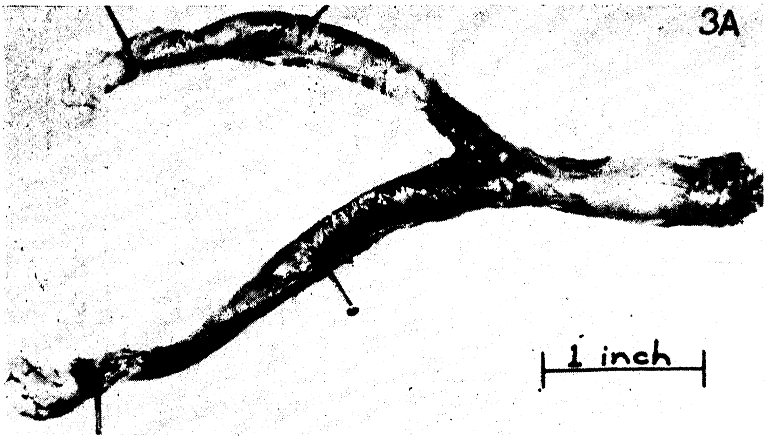


FIG. 2A. HIGH POWER PHOTOMICROGRAPH of pars anterior of *dog 1* to illustrate presence of acidophiles (dark cells); basophiles (dark gray cells); and chromophobes (lighter and smaller cells). Heidenhain azan method of staining.

FIG. 2B. PHOTOMICROGRAPH AT SAME MAGNIFICATION as 2 A taken from a portion of the pars anterior remnant of *dog 3*. A decrease in the granules in both the acidophiles and basophiles is noted. Heidenhain azan method of staining.

Dog 3 was an immature female which developed to sexual maturity postoperatively; however this development was definitely delayed (see fig. 3A). Necropsy performed 16 months after operation revealed that the amount of pars anterior tissue remaining constituted about one sixth of the original total volume. Although some fully granulated cells were visible, a majority of the basophiles were found to be partially or totally degranulated.[2] A slight reduction in the number of acidophiles had occurred.



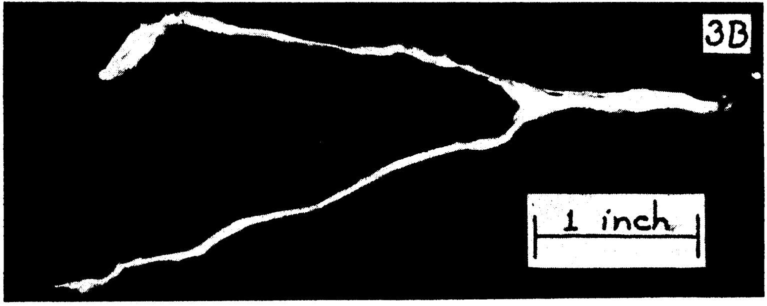


FIG. 3A. PHOTOGRAPH OF THE OVARIES AND GENITAL TRACT of *dog 3* which demonstrates complete development subsequent to the stalk removal procedure.

FIG. 3B. PHOTOGRAPH OF OVARIES AND GENITAL TRACT of *dog 8* which demonstrates the complete failure in development subsequent to the stalk removal procedure.

Gross inspection of the anterior lobe tissue in *dogs 4* and *5* showed that practically the entire pars anterior had persisted following the stalk removal procedure. The postoperative periods for these two dogs were 9 and 8 months, respectively. These tissues gave histological evidence of a slight reduction in the numbers of basophile cells when compared to normal anterior lobe tissue. The acidophiles were numerous and well granulated. Chromophobic cells did not appear to deviate from those seen in normal tissue.

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[2] The appearance of the basophiles described above may be correlated, in part, with the findings of R. Gregory and G. A. Drager, Federation Proc. **6**: 116, 1947, in which they reported that the basophiles increased in size and became degranulated following total pancreatectomy. This dog survived total pancreatectomy four weeks.

The female *dog 6* was retained for 10 months following the hypophysial stalk removal procedure and at the time of termination showed that approximately one third of the normal amount of anterior lobe tissue had remained distally. Its microscopic appearance was such that the cell population did not vary noticeably from similar fields seen in an intact gland. Basophiles, filled with granules, were common in all fields and acidophilic cells were numerous and well granulated. No change could be noted in the number or character of the chromophobe cells.

Failure of development of or regression of sex organs. Of the remaining three females, *dog 7* was sexually mature at operation, and necropsy eight months later showed regression of all sex organs. The other two, *dogs 8* and *9*, were sexually immature at the time of the hypophysectomy procedure, and retained infantile reproductive systems throughout the postoperative period of 10 months (see fig. 3B). The quantity of anterior lobe tissue viable at autopsy and later verified by microscopic observation revealed an almost total absence of granulated basophiles as well as a marked reduction in the partially granular and agranular stages of this cell type. Although fully developed acidophiles were present, there was a definite reduction in their number. Chromophobe cells predominated in all areas of the viable gland tissue, and their appearance was compatible with those seen in an intact gland.

DISCUSSION

The relation of the size of the isolated pars anterior remnant to the retention of sex functions. It is a common, and correct, practice to relate basal, reserve and remnantal endocrine functions with 1) the amount of a particular gland tissue which can be removed experimentally without producing a demonstrable deficit and 2) the over-all size of a tissue remnant which is able to prevent an extirpation deficit from being total. All of the endocrine glands that have been examined according to these criteria have been found to have tremendous reserves in terms of tissue mass. It also has been demonstrated that the smallest anatomical remnant must not be ignored when a total deficit is being assayed. The work of Smith on rats (5) gives ample evidence that these criteria obtain between gonadotropic function and the pars anterior tissue of the adenohypophysis.

It nevertheless cannot be assumed that the mere presence of a large viable tissue remnant ensures continuance of function. That a large pars anterior tissue mass may be viable while at the same time be non-functional, with respect to its control over sex functions, is well-illustrated in *dogs 7* and *8* of this series. *Dog 7* failed to develop sexually in spite of the fact that it had about as much recognizable viable pars anterior tissue as *dog 2* and more than did *dog 3*. In other words, in terms of the estimation of relative size, as judged by inspection of representative serial sections stained by a non-differential stain, such as cresyl violet, one would have expected the same functional results for both *dogs 2* and *7*.

In the presence of a deficit, it is accordingly relatively futile to speak in terms of weight or estimated size of a viable remnant of pars anterior tissue.

Relation of the specific cytology of the isolated pars anterior tissue to the retention of sex functions. The cytology of the mammalian pituitary has been subjected to critical analyses, associated with indirect functional studies, by numerous investigators in the attempt to correlate a specific function to specific anatomical cells. As a result, gonadotropic hormone production has long been believed to be a specific function of the basophiles found in the pars anterior component of the adenohypophysis. In the present studies, the cytology of the viable isolated pars anterior tissue did not deviate markedly from the normal in those animals in which sex functions were retained. In those in which sex deficits were in evidence, the cytology of the basophiles deviated considerably from the normal. Accordingly, our results are entirely compatible with the accepted view that the basophiles are the cells which elaborate the gonadotropic principles (Severinghaus, 6; Heinbecker and Rolf, 7; Dawson, 8).

Independence of the pars anterior from any possible direct hypothalamic influence. It is apparent from this series of animals that normal sex functions may be retained in the dog following isolation of a distal portion of the pars anterior from any possible direct hypothalamic influence. These findings are in accord with those presented by Keller and Hamilton in dogs (9), Brooks and Lambert in male rabbits (10), Dempsey in guinea pigs (11) and Dempsey and Uotila in rats (12). Our present experiments, other than being made on dogs rather than on rodents, are distinctive only in extending the foregoing observations pertaining to stalk section to those following complete isolation of a *distal portion* of the pars anterior. In this respect they are somewhat equivalent to the experiments of Greep in rats (13). It of necessity follows that interference in sex function following stalk section procedures or by hypothalamic lesions must be due to a factor or factors other than to the interruption of an essential direct hypothalamic innervation of the pars anterior.

SUMMARY

The distal portion of the pars anterior was isolated by the surgical procedure of removing the hypophysial stalk tissue and the proximal portion of the pars anterior in nine dogs.

Sexual functions remained completely intact in two dogs as evidenced by their ability to reproduce. When judged by gross and histological studies, the ovaries and genital tracts remained normal in four others of the group. In the three remaining dogs the ovaries and genital tracts, as revealed by histological studies, were markedly atrophied.

There was no correlation between retention of sex functions and the size of the isolated mass of pars anterior tissue remaining. Two of the dogs, in which there was obvious sex regression, had as much and in instances more viable pars anterior tissue remaining than did the dogs which retained sex functions.

There was a definite correlation between retention of sex functions and the cytology of the isolated pars anterior remnant. In those instances where functional deficits were demonstrated, both the basophiles and acidophiles were reduced in numbers and the basophiles which were present did not stain normally.

REFERENCES

1. Keller, A. D., W. E. Lawrence, and C. B. Blair. Arch. Path. **40**: 289, 1945.
2. Keller, A. D., and C. G. Breckenridge. This Journal **150**: 222, 1947.
3. Rasmussen, A. T. Proc. Soc. Exper. Biol. & Med. **34**: 760, 1936.
4. Dawson, A. B. Stain. Tech. **4**: 133, 1939.
5. Smith, P. E. Anat. Rec. **52**: 191, 1932.
6. Severinghaus, A. E. Assoc. Res. in Nerv. & Ment. Dis. XVII, 93, 1938.
7. Heinbecker, P., H. L. White, and D. Rolf. This Journal **141**: 566, 1944.
8. Dawson, A. B. Am. J. Anat. **78**: 347, 1946.
9. Keller, A. D., and J. W. Hamilton. This Journal **119**: 349, 1937.
10. Brooks, C. M., and E. F. Lambert. This Journal **128**: 57, 1939.
11. Dempsey, E. W. This Journal **126**: 758, 1939.
12. Dempsey, E. W., and U. U. Uotila. Endocrinology **27**: 573, 1940.
13. Greep, R. O. Proc. Soc. Exper. Biol. & Med. **34**: 754, 1936.

EFFECT OF ADRENALECTOMY UPON THE TOLERANCE OF THE EVISCERATED RAT FOR INTRAVENOUSLY ADMINISTERED GLUCOSE

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In these experiments eviscerated and adrenalectomized-eviscerated rats were given continuous intravenous infusions of glucose with and without insulin for periods of 4 and 24 hours. At the end of 4 hours the average values for blood glucose were significantly lower in the adrenalectomized than in the non-adrenalectomized series at each glucose load and insulin dose. Within the 24-hour period the adrenalectomized animals removed glucose from the blood more rapidly than nonadrenalectomized animals when little or no insulin was given and the glucose loads remained low. When the comparisons were made at higher levels of insulin dosage and of glucose load the order of tolerance was reversed in that the adrenalectomized animals had higher average terminal values for blood glucose than the non-adrenalectomized rats.

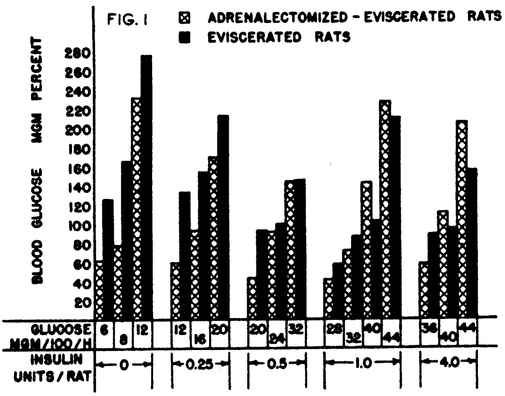
METHODS

Male rats of the Sprague-Dawley strain were fed Friskies Dog Cubes. At a weight of 185 to 205 grams, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 (±2) grams they were anesthetized (intraperitoneal injection of 18 mgm. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure of Ingle and Griffith (1). The adrenal glands were ligated and removed in the same operation. Hemostasis was attained by applying a gelatin sponge (Gelfoam, Upjohn) saturated with a solution of thrombin to the stumps of the oesophagus, colon, ligated vessels and between the muscle and the skin when the incisions were closed.

Intravenous injections of solutions containing 0.9 per cent sodium chloride and varying concentrations of glucose (C.P. Dextrose, Merck) with and without crystalline zinc insulin (Lilly) were made by two continuous injection machines which delivered fluid from each syringe at the rate of 20 cc. in 24 hours. The glucose load is expressed as milligrams of glucose per 100 grams of rat per hour (mgm/100/h). Syringes of the Luer-Lok type (Becton-Dickinson) were selected to deliver 20 cc. with a stroke of 65 mm. Six syringes were operated by each machine. The machines were powered by synchronous motors, and the reduction of motion was achieved by a precision-built system of gears which exactly controlled the rate of injection.

The infusions were made into the saphenous vein of the right hind leg and were started within five minutes following the removal of the liver. The animals were secured in a supine position on an animal board and were enclosed in a cabinet with the temperature constant at 26.5 (±0.5) degrees C. The temperature of the room was maintained at 74 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation. The more rigid control of temperature has decreased the variability of results and has slightly increased the tolerance for glucose over average values reported earlier (2). As the temperature rises, the insulin sensitivity of the animal is increased. A change of as little as one degree C. significantly changes the glucose requirement of the insulin-treated eviscerate rat. A complete control of temperature and humidity would be desirable.

The analyses of blood glucose were made by the method of Miller and Van Slyke (4). In addition to glucose, this method measures small amounts of non-fermentable reducing substances which accumulate in the blood of eviscerate rats. Samples of blood were taken from the jugular vein at the end of 4 hours of infusion in experiment 1 and at the end of 24 hours of infusion in experiment 2. The urine was tested for glucose at the end of each experiment but was negative (less than 40 mgm. reducing substances) for each animal.



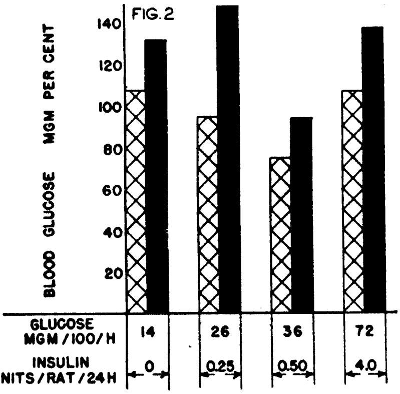


Fig. 1. Average values for blood glucose at the end of 4 hours of continuous intravenous infusion. Eviscerated rats (■) and adrenalectomized-eviscerated (⊠) rats.

Fig. 2. Average values for blood glucose at the end of 24 hours of continuous intravenous infusion.

EXPERIMENTS AND RESULTS

In experiment 1 (fig. 1) 4 groups of rats having 20 pairs of rats in each group were infused with glucose for a period of 4 hours. One rat of each pair was adrenalectomized. The first group was given a glucose load of 14/100/h. without insulin; the second group was given a glucose load of 26/100/h. with insulin at the rate of 0.25 units per 24 hours per rat; the third group was given a glucose load of 36/100/h. with insulin at the rate of 0.5 units per 24 hours per rat; and the fourth group was given a glucose load of 72/100/h. with insulin at the rate of 4 units per 24 hours per rat. In each group the average level of blood glucose at the end of 4 hours was significantly lower in the adrenalectomized as compared to the non-adrenalectomized animals.

In experiment 2 (fig. 2) the tolerance for glucose was determined over a 24-hour period. Twelve pairs of rats were tested in each group. Three groups of rats without insulin were studied at glucose loads of 6, 8 and 12/100/h.; at an insulin dose of 0.25 units 3 groups of rats were tested at 12, 16 and 20/100/h.; at an insulin dose of 0.5 units 3 groups of rats were tested at 20, 24 and 32/100/h.; at an insulin dose of 1.0 units 4 groups of rats were tested at 28, 32, 40 and 44/100/h.; and at an insulin dose of 4 units 3 groups of rats were tested at 36, 40 and 44/100/h.

In eviscerated rats without insulin and at a dose of 0.25 units of insulin the adrenalectomized rat removed glucose from its blood at a more rapid rate than did the non-adrenalectomized rats as indicated by the significantly lower terminal averages for blood glucose. At higher insulin dosages the average values for blood glucose in the adrenalectomized rats remained lower when the glucose loads were relatively low for that insulin dose. At glucose loads of 40 and 44/100/h. the adrenalectomized animals showed higher average rises in blood glucose than the non-adrenalectomized animals.

DISCUSSION

This study provides additional evidence that the adrenal cortical hormones affect carbohydrate metabolism in the absence of the liver. Russell (6) and Roberts (5) have each observed that adrenalectomy accelerates the rate at which glucose disappears from the blood of the eviscerated rat. In an earlier study from this laboratory (2) it was shown that within a 24-hour period the administration of large amounts of adrenal cortex extract definitely depressed the tolerance for glucose in those rats which received insulin. In the absence of insulin the effect of adrenal cortex extract upon glucose tolerance was either absent or questionable. For this reason it was anticipated that the effect of adrenalectomy would be the greatest in those animals which were treated with insulin. The contrary was true for the greatest effect of adrenalectomy upon glucose tolerance occurred in animals which received little or no insulin. The following rationalization of these apparently paradoxical results is offered without the assumption that it is the only possible explanation.

The utilization (storage, oxidation and conversion) of glucose by the eviscerated rat without insulin over a 24-hour period is very small, about 4 mgm. of glucose per 100 grams of rat per hour. Possibly this low level of glucose utilization is physiologically basal and cannot be inhibited further by agents such as an excess of the adrenal cortical hormones. Glucose utilization could, however, be shifted in the upward direction by conditions such as adrenal cortical insufficiency which favor increased glucose utilization. In the presence of optimal amounts of insulin the inactive eviscerated rat can utilize about 40 mgm. of glucose per 100 grams of rat per hour. Possibly this rate of utilization should be regarded as a physiological ceiling for the inactive eviscerated rat which cannot be increased by adrenal cortical insufficiency. Factors which favor inhibition of glucose utilization such as the adrenal cortical hormones could readily manifest their effects upon the ceiling of utilization but not upon basal utilization. To continue speculation, why is the utilization of glucose apparently less in the adrenalectomized-eviscerated rat when the glucose load is high? This may be the result of the comparative inefficiency with which the adrenalectomized animal carries out any metabolic process when it is pushed to its physiological limit. The adrenalectomized-eviscerated animal is approaching death more rapidly than a similar animal which is supplied with the cortical hormones. It is not necessary to assume the existence of a physiological ceiling for the utilization of glucose. It can be postulated that the secondary inefficiencies of the adrenally insufficient animal completely mask the more primary effect of adrenal cortical insufficiency at high glucose loads. An analogical situation obtains in respect to the ability of the adrenalectomized dog to excrete sodium and chloride. At the usual levels of sodium chloride intake the adrenalectomized animal excretes these ions more rapidly than normal. However, when the intake of sodium chloride is increased to the limit of tolerance the ability of the adrenalectomized animal to excrete the excess of these ions is less than normal (3).

The above rationalization of the results from the 24-hour tests is weakened by the differences in results of the 4-hour and 24-hour tests. The administration of large amounts of adrenal cortex extract to the eviscerated rat (2) did not have any significant effect within a period of 4 hours whereas the effects of adrenalectomy were evident in animals at each glucose load either with or without insulin. Possibly within a period of 4 hours the major effect of adrenalectomy is to release the inhibitory effect of the cortical hormones upon glucose utilization prior to the onset of the secondary inefficiency of the adrenalectomized-eviscerated rat which develops at a later time as the animal approaches death.

The results of this study give some support to the conclusion that the effect of the adrenal cortex upon carbohydrate utilization is not dependent upon the presence of insulin. Although the pancreas has been completely removed from the eviscerated rat, it is still possible, however, that some insulin remains in the blood and tissues. Our data do not exclude the possibility that in the eviscerated rat the effect of cortical hormone deficiency and excess upon the tolerance for glucose is based upon the change in the rate of gluconeogenesis in the kidney and other extra-hepatic tissues. We recognize the possibility that adrenal medullary insufficiency may have been a factor in causing the differences between the adrenalectomized and nonadrenalectomized rat.

SUMMARY

Male rats (185–205 gram) of the Sprague-Dawley strain were caused to develop a collateral circulation by ligation of the inferior vena cava. At a weight of 250 ± 2 grams the animals were anesthetized (cyclopal), and all of the intra-abdominal organs were removed except the kidneys. The adrenal glands were removed in one animal of each pair in the same operation. Infusions into the saphenous vein were made by continuous injection machines which delivered fluid at the rate of 20 cc. in 24 hours per rat. The level of blood glucose at the end of the injection period was the index of glucose tolerance.

In experiment 1, 4 groups of rats having 20 pairs of rats in each group were

infused with glucose with and without insulin for a period of 4 hours. One rat of each pair was adrenalectomized. At each glucose load with and without insulin the average level of blood glucose at the end of 4 hours was significantly lower in the adrenalectomized than in the non-adrenalectomized animals.

In experiment 2 the tolerance for glucose was determined over a 24-hour period. Sixteen groups of rats having 12 pairs of animals in each group were infused with different loads of glucose with and without insulin. Within the 24-hour period the adrenalectomized animals removed glucose from the blood more rapidly than the non-adrenalectomized animals when little or no insulin was given and the glucose loads remained low. At the higher levels of insulin dosage and glucose loads the order of tolerance was reversed and the adrenalectomized animals had higher average terminal values for blood glucose than the non-adrenalectomized rats.

REFERENCES

1. Ingle, D. J. and J. Q. Griffith. The rat in laboratory investigation. Philadelphia, J. B. Lippincott Co., 1942, Chapter 16.
2. Ingle, D. J., M. C. Prestrud, J. E. Nezamis and M. H. Kuizenga. This Journal **150:** 423, 1947.
3. Kottke, F. J., C. H. Code and E. H. Wood. This Journal **136:** 229, 1942.
4. Miller, B. F. and D. D. Van Slyke. J. Biol. Chem. **114:** 583, 1936.
5. Roberts, S. Endocrinology **39:** 90, 1946.
6. Russell, J. A. This Journal **140:** 98, 1943.

EFFECT OF ADRENALECTOMY UPON THE URINARY EXCRETION OF GLUCOSE AND NONPROTEIN NITROGEN IN THE PARTIALLY DEPANCREATIZED, FORCE-FED RAT

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Removal of the adrenal cortex from depancreatized animals results in amelioration of the symptoms of diabetes, whereas administration of adrenal cortex extracts and C_{11}-oxygenated cortical steroids cause exacerbation of the symptoms of diabetes. Earlier studies (1, 2) on the mechanism of the relationship between adrenal cortical function and carbohydrate metabolism showed that the reduction of glycosuria following adrenalectomy was accompanied by a decrease in urinary nitrogen, whereas the administration of diabetogenic amounts of the cortical hormones caused an increased breakdown of protein as evidenced by increased nitrogen loss. It was postulated that the principal effect of the cortical hormones upon organic metabolism was upon gluconeogenesis from protein. Subsequent studies (3–6) have indicated that the cortical hormones also affect the utilization of carbohydrate. The utilization of carbohydrate is increased by cortical insufficiency and depressed by an excess of the cortical hormones.

An appraisal of the role of cortical insufficiency in the decrease of urinary nitrogen which follows adrenalectomy in the diabetic animal should include the two following considerations: *a*) The diabetic animal characteristically eats larger than normal amounts of food and the untreated adrenalectomized animal eats smaller than normal amounts of food. The change in protein intake must be responsible for a part of the change in nitrogen excretion which follows adrenalectomy in the diabetic animal. *b*) Nitrogen loss is characteristically higher than normal in the diabetic animal and amelioration of diabetes is followed by restoration of a normal nitrogen balance. The possibility that the decrease in nitrogen loss following adrenalectomy in the depancreatized animal is secondary to improvement in glucose utilization deserves consideration.

In these experiments partially depancreatized rats were given a constant food intake by stomach tube. Following adrenalectomy there was a dissociation between the changes in urinary glucose and urinary nonprotein nitrogen. The decrease in urinary nitrogen was much too small to account for the decrease in glycosuria as being due to a decrease in gluconeogenesis from protein.

METHODS

Male rats of the Sprague-Dawley strain which were completely free from infection were used. The stock diet was Friskies Dog Cubes. At a weight of approximately 300 grams the animals were partially depancreatized by the method of Ingle and Griffith (7). After diabetes was established all of the animals were placed in metabolism cages and were fed a medium carbohydrate diet made according to table 1. All of the animals were force-fed by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The techniques and diet were modifications of those described by Reinecke, Ball and Samuels (8). During the period of adaptation to forced feeding, the amount of diet was increased gradually to prevent the development of 'food-shock.' The animals were brought to a full feeding of 26 cc. of diet per rat per day on the fifth day. The animals were kept in an air-conditioned room with temperature at 74 to 78° F. and humidity at 30 to 35 per cent of saturation. Twenty-four-hour samples of urine were collected at the same hour (8:00 to 8:30 A.M.) and were preserved with thymol and added citric acid (one gram per sample) to insure the acidity of the urine for nitrogen analyses. Urine glucose was determined by the method of Benedict (9), and the determination of urinary non-protein nitrogen was by the micro-Kjeldahl procedure as follows: proteins were precipitated as the salts of tungstic acid by the Folin-Wu procedure. The organic matter was oxidized by sulfuric acid and hydrogen peroxide. The ammonia was distilled off into a standard acid solution and titrated with standard base.

TABLE 1. MEDIUM CARBOHYDRATE DIET

Constituent	Grams
Cellu flour (Chicago Dietetic Supply)	120
Osborne & Mendel salt mixture	40
Diet yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vitamin K (2-methyl-1,4-naphthoquinone)	100 mgm.
Mazola oil	200
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Water to make total of	2000 cc.

Following a control period all of the animals were adrenalectomized by the method of Ingle and Griffith (7). Sterile technique was used and infections were successfully avoided. The animals were given a one per cent sodium chloride solution to drink throughout the entire experiment. Beef adrenal extract (Upjohn) representing 40 grams of gland per cc. was used. It was free from alcohol.

EXPERIMENTS AND RESULTS

In experiment 1 (fig. 1) eight partially depancreatized, mildly diabetic rats were adrenalectomized following a control period of seven days. Following adrenalectomy they were maintained by drinking saline for 14 days. At this time cortical extract was administered according to the following: one cc. per day for seven days, 2 cc. per day for seven days and 4 cc. per day for seven days. At the same time, seven normal rats were studied under the same conditions of forced feeding. The average daily value (231 mgm.) for urinary N.P.N. is shown for comparative purposes in figure 1.

Prior to adrenalectomy the diabetic rats excreted higher than normal amounts of nitrogen and an average of more than 3 grams of glucose per day. Following adrenalectomy the excretion of nitrogen was depressed for one day, but by the third day it increased above the preoperative level. This rise occurred at a time when the level of urinary glucose was falling. There was a secondary fall in urinary nitrogen below the preadrenalectomy level, but it did not fall below the average for normal rats during the first 14 days. The glycosuria was abolished for each rat by the end of this period. During the administration of one cc. and 2 cc. of cortical extract per day the average level of urinary nitrogen fell below the normal average although glycosuria was re-established. When the amount of cortical extract was increased to 4 cc. per day the values for both urinary nitrogen and glucose were elevated to preadrenalectomy levels and above.

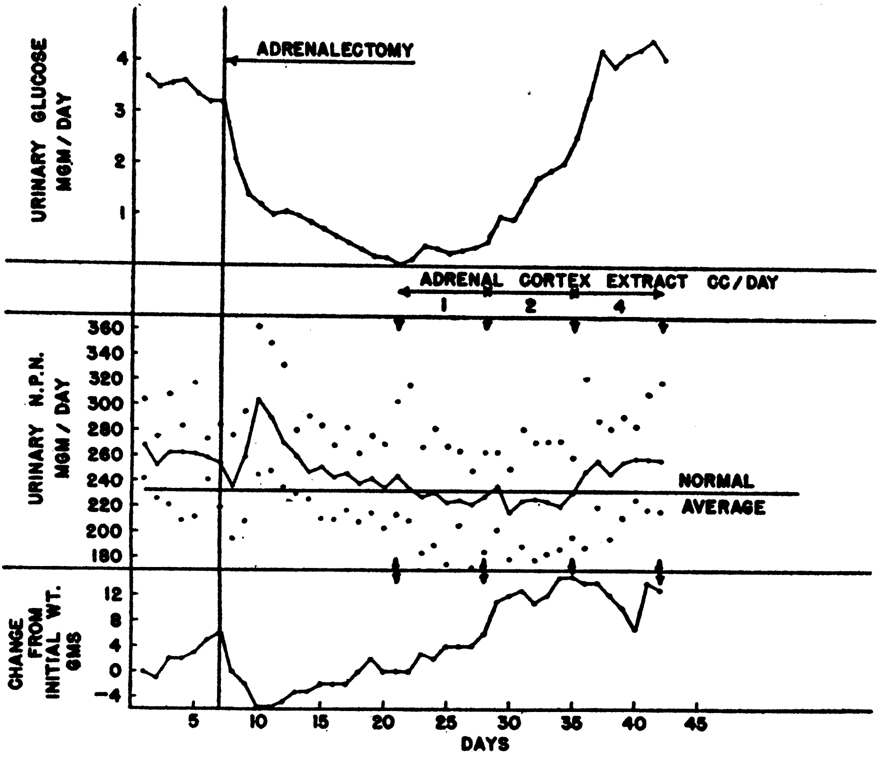

Fig. 1. Partially depancreatized, forced-fed rats. Average values for eight animals. The range of individual values is given for urinary N.P.N.

In experiment 2 an attempt was made to determine the effects of adrenalectomy upon severely diabetic rats. Six extensively depancreatized rats were adrenalectomized but only three of them survived the 14-day period during which treatment was limited to the drinking of saline. The results on the three animals which survived were in accord with the results of experiment 1. Prior to adrenalectomy these animals excreted an average of 4.5 grams of glucose and 283 mgm. of nonprotein nitrogen daily. Following adrenalectomy the glycosuria fell to an average daily value of 2.3 grams by the end of 14 days, and the corresponding value for urinary nitrogen was 252 mgm. Again, there was a decrease in urinary nitrogen immediately following adrenalectomy and a subsequent temporary rise. During treatment with one cc. and 2 cc. of cortical extract per day the glycosuria increased, while the level of urinary nitrogen decreased. During treatment with 4 cc. per day the levels of urinary nitrogen and glucose were elevated to preadrenalectomy levels. These data are not charted.

DISCUSSION

Janes, Dawson and Myers (10) found that amelioration of alloxan diabetes by adrenalectomy in rats which ate ad libitum was associated with a lowered food intake and that nonadrenalectomized, diabetic rats which were limited to the same food intake showed a similar decrease in glycosuria. The present study confirms the observations of Ingle and Thorn (5) in showing that adrenalectomy caused an amelioration of pancreatic diabetes in rats which had a normal caloric intake sustained by tube feeding. The intestinal absorption of carbohydrate in these animals was complete.

The decrease in urinary glucose following adrenalectomy (approximately 3.5 grams) cannot be accounted for in terms of decreased gluconeogenesis from protein when changes in urinary nonprotein nitrogen are used as an index of the extent of conversion. Even if it were assumed that all of the carbon of the catabolized protein was converted to carbohydrate prior to the adrenalectomy and none following adrenalectomy the change in urinary glucose was greater. There was a postadrenalectomy rise in urinary nitrogen which coincided with a period of rapid fall in the glycosuria. An additional dissociation in the direction of change in urinary glucose and nitrogen occurred during the injection of one cc. and 2 cc. daily of cortical extract when the level of urinary nitrogen decreased and glycosuria was reinduced up to average values of more than 2 grams per day.

These results are in agreement with experiments on cortical hormone overdosage in partially depancreatized (5) and normal (11) force-fed rats, in which it was shown that the glucose loss is too great to be accounted for in terms of increased protein breakdown.

None of our experiments has tested the possibility that the cortical hormones affect gluconeogenesis from fat. However, in the liverless rat glucose tolerance is increased by adrenal cortical insufficiency (12) and is depressed by cortical hormone excess (13). In our opinion the hypothesis which most nearly meets the experimental facts is the general explanation advanced by Evans (6) that the cortical hormones affect some mechanism which spares carbohydrate. Accordingly, the adrenalectomized, diabetic animal regains ability to utilize some of the carbohydrate which was formerly wasted into the urine, and cortical hormone overdosage interferes with the utilization of carbohydrate. The phase of utilization involved may or may not be that of oxidation. The manner in which this effect of cortical insufficiency and excess relates to the role of the cortical hormones in body economy is not known.

There was some decrease in the level of urinary nitrogen following adrenalectomy but it did not fall below the average for normal animals during the 14-day postoperative period when no hormone was given. Diabetic animals characteristically excrete greater than normal amounts of nitrogen but a normal nitrogen balance is established when the glycosuria is controlled by insulin. Was the decrease in urinary nitrogen following adrenalectomy in these diabetic animals secondary to an improved utilization of glucose or does it reflect a more primary function of the cortical hormones? It has been generally considered that protein catabolism is decreased by cortical insufficiency but other studies from this laboratory (14, 15) have indicated that the force-fed, saline-treated, adrenalectomized rat is able to catabolize protein in a normal manner. Such animals do fail to mobilize their tissue proteins at a normal rate during a severe stress (15) unless they are sustained with cortical hormones.

When the administration of cortical extract was started after 14 days of saline treatment the nitrogen balance became more strongly positive. Did this represent an effect of the cortical extract? It is possible that this small change would have occurred without the change in treatment. There was a corresponding increase in body weight (fig. 1). It has been observed on several occasions in this laboratory that the force-fed, adrenalectomized rat does not gain weight quite as rapidly as the nonadrenalectomized animal. It is possible that the cortical hormone is essential for an optimal rate of anabolism although this is contrary to the generally accepted concept that the cortical hormones stimulate catabolic processes. This may only be true of cortical hormone overdosage and is not necessarily incompatible with the assumption that under some conditions physiological amounts of these hormones may support anabolism.

The amounts of beef adrenal extract required to restore the glycosuria of the adrenalectomized-depancreatized rats to its preadrenalectomy level are large when compared to doses adequate to sustain life (16). From these data (fig. 1) it can be estimated that prior to adrenalectomy the adrenal cortices of these animals secreted the activity equivalent of 3 to 4 cc. of extract per day. It is probable that the utilization of the endogenous secretion is more efficient than the intermittent injections of cortical extract. There are possible fallacies in all methods of estimating the secretory capacity of the adrenal cortices but all methods support the conclusion that very large amounts of cortical extracts are required to fully replace the functional activity of the intact adrenal glands (17).

SUMMARY

Partially depancreatized male rats of the Sprague-Dawley strain were force-fed a medium carbohydrate diet and were given a one per cent solution of sodium chloride to drink throughout the experiment. They excreted an average of 3.5 to 4.0 grams of glucose per day and the nitrogen loss was greater than normal. Following adrenalectomy there was a sharp increase in urinary nitrogen by the

third day, which coincided with a fall in the level of urinary glucose. The glycosuria disappeared and the urinary nitrogen gradually fell to normal values, but not below, during the 14 days that the animals were maintained without cortical extract. During the administration of one cc. and 2 cc. of beef adrenal extract per day the average level of urinary nitrogen fell below the normal average, although glycosuria was re-established. When the amount of cortical extract was increased to 4 cc. per day the values for both urinary nitrogen and glucose were elevated to preadrenalectomy levels and above. A decrease in gluconeogenesis from protein, as evidenced by the change in urinary nitrogen, could account for less glucose than the actual decrease of 3500 mgm. per day in urinary glucose (exp. 1). Adrenalectomy either caused a decrease in gluconeogenesis from fat or the animals regained the ability to utilize the dietary carbohydrate which they wasted by excretion prior to adrenalectomy.

REFERENCES

1. Long, C. N. H. and F. D. W. Lukens. J. Exp. Med. **63**: 465, 1936.
2. Long, C. N. H. Transactions and studies of the College of Physicians of Philadelphia. **7**: 21, 1939.
3. Thorn, G. W., G. F. Koepf, R. A. Lewis and E. F. Olsen. J. Clin. Invest. **19**: 813, 1940.
4. Long, C. N. H., B. Katzin and E. G. Fry. Endocrinology **26**: 309, 1940.
5. Ingle, D. J. and G. W. Thorn. This Journal **132**: 670, 1941.
6. Evans, G. Endocrinology **29**: 731, 1941.
7. Ingle, D. J. and J. Q. Griffith. The rat in Laboratory Investigation. Philadelphia: J. B. Lippincott Co., 1942 Chapter 16.
8. Reinecke, R. M., H. A. Ball and L. T. Samuels. Proc. Soc. Exp. Biol. Med. **41**: 44, 1939.
9. Benedict, S. R. J. Am. Med. Assoc. **57**: 1193, 1911.
10. James, R. G., H. Dawson and L. Meyers. This Journal **145**: 538, 1946.
11. Ingle, D. J., R. Sheppard, J. S. Evans and M. H. Kuizenga. Endocrinology **37**: 341, 1945.
12. Ingle, D. J. and J. E. Nezamis. This Journal. In press.
13. Ingle, D. J., M. C. Prestrud, J. E. Nezamis and M. H. Kuizenga. This Journal **150**: 423, 1947.
14. Ingle, D. J. and E. A. Oberle. This Journal **147**: 222, 1946.
15. Ingle, D. J., E. O. Ward and M. H. Kuizenga. This Journal **149**: 510, 1947.
16. Cartland, G. F. and M. H. Kuizenga. This Journal **117**: 678, 1936.
17. Ingle, D. J. J. Clin. Endocrinol. **4**: 208, 1944.

INFLUENCE OF A PREVIOUS INJECTION OF EPINEPHRINE UPON THE DIABETOGENIC EFFECT OF ALLOXAN IN RABBITS

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During the course of studies on the action of alloxan on rabbits, an attempt was made to prevent the well-known noxious action of this drug on the kidney by interrupting the renal circulation for about six minutes at the time of the alloxan injection. It was found that rabbits thus treated either did not become diabetic or developed an extremely mild diabetes. One explanation for the protective effect observed is that our operative procedure resulted in an epinephrine discharge. Kass and Weisbren (1) have recently described a similar protective effect in rats by injection of epinephrine previous to the injection of alloxan. This prompted us to investigate the influence of a previous injection of epinephrine upon the diabetogenic effect of alloxan on rabbits.

METHODS

Twenty-two rabbits weighing 950 to 1925 grams (average 1400 grams) were given intravenous injections of commonly used diabetogenic doses of alloxan (170 to 204 mgm. per kilo of body weight) preceded by injections of epinephrine. Those that survived longer than 24 hours were kept in metabolic cages and their urine was collected daily and tested for dextrose (Benedict). Blood sugar and occasionally blood urea were measured in blood samples from the marginal ear vein by means of the Leitz electrophotometer (slightly modified Folin and Wu and Karr methods). Blood sugar figures given below are mgm. of dextrose per 100 cc. of blood. Dextrose tolerance tests were performed after 16 to 20 hours fast by measuring blood sugar level before, and two hours after, the injection of 4 cc. of 50 per cent dextrose solution per kilo of body weight. When the fasting blood sugar level was lower than 150 and the two-hour figure was not higher than the first one, the result was considered as showing the absence of a diabetic disturbance; when the two-hour figure was higher than 150, and also distinctly higher than the fasting one, the result was considered as showing the presence of a diabetic disturbance; other results would be considered doubtful. Further details concerning the experimental conditions are given in the tables.

RESULTS AND DISCUSSION

The results of our experiments are given in tables 1, 2 and 3. Out of a total number of 22 animals treated with epinephrine-alloxan, as detailed above, 11 survived longer than 24 hours (table 1). *At a time when a large proportion of*

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TABLE 1. DATA CONCERNING 11 RABBITS (OUT OF A SERIES OF 22) THAT SURVIVED NOT LESS THAN 24 HOURS AFTER AN INTRAVENOUS INJECTION OF A CURRENT DIABETOGENIC DOSE OF ALLOXAN PRECEDED BY AN INTRAMUSCULAR INJECTION OF A DOSE OF 200 MICROGRAMS PER KGM. OF EPINEPHRINE

RABBIT NO.	WEIGHT	ALLOXAN PER KGM.	INTERVAL BETWEEN INJECTIONS	LENGTH OF FASTING PRIOR TO INJECTIONS	TIME OF ONSET OF HYPOGLYCEMIA AFTER ALLOXAN INJECTION[1]	GLYCOSURIA LATER THAN 24 HOURS AFTER ALLOXAN INJECTION	FASTING BLOOD SUGAR: 24 hours after alloxan injection	FASTING BLOOD SUGAR: 48 hours after alloxan injection	FASTING BLOOD SUGAR: Highest value found after 48 hours	SURVIVAL PERIOD	CAUSE OF DEATH[2]
	grams	*mgm.*	*mins.*	*hours*	*hours*		*mgm/ 100 cc.*	*mgm/ 100 cc.*	*mgm/100 cc.*		
1	1750	185	60	24	2	–	77		126 (12th) day	7 months	Killed
4	1650	200	60	24	4		90			less than 48 hours	Liver damage
5	1750	180	60	none	8	–	117	100	100 (8th) day	12 months	Killed
6	1240	200	60	24	3	–	37	160	97 (4th) day	4 days	Liver damage
8	1150	200	60	24	2		62			less than 48 hours	Uremia
9	1350	170	60	24	2	+		136	322 (4th) day	4 days	Killed
13	1270	200	20	none	7		408			less than 48 hours	Uremia
14	1320	200	20	none	5½	–	410			8 days	Liver damage
19	1635	180	60	16	5	–	124		124 (6th) day	12 months	Killed
20	1225	180	80	16	7	–	65	50		49 hours	Liver damage
21	1650	180	80	none		–	171	132	109 (5th) day	12 months	Killed

[1] *Rabbit 21* did not show hypoglycemia at any time; it was given dextrose injections, beginning five hours after alloxan. Dextrose injections, beginning 2 to 3 hours after alloxan (before hypoglycemia was ascertained), were given to *rabbits 13, 14, 19* and *20*. Dextrose injections, beginning after hypoglycemia was ascertained, were given to *rabbits 1, 4, 5, 6, 8* and *9*.

[2] For *rabbits 4, 6, 14* and *20* liver damage is given as the cause of death because in all these animals extremely severe liver necrosis was found by histological examination. *Rabbits 8* and *13* were not subject to postmortem examination; uremia is given as the cause of their death because the 24-hour blood urea was 103 mgm.% for *no. 8* and 140 mgm.% for *no. 13;* liver damage, as contributory factor, is of course probable.

commonly alloxanized rabbits already show an unmistakable diabetic hyperglycemia, namely 24 hours after the alloxan injection (2, 3, 4), in only 3 of our 11 surviving animals was blood sugar higher than 125. Eight of our rabbits survived 48 hours or longer. At a time when nearly all commonly alloxanized rabbits already show unmistakable diabetic hyperglycemia, namely 48 hours after the alloxan injection (2, 3, 4), blood sugar was measured in five animals and in none was it found to be higher than 160; *rabbit no. 9*, with a 48-hour blood sugar figure of only 136, showed glycosuria on the third and a blood sugar of 322 on the fourth day. None of the other six animals surviving longer than 48 hours (*nos. 1, 5, 6, 14, 19,* and *21*) ever showed either glycosuria or fasting hyperglycemia later than 48 hours after the alloxan injection.

Sugar tolerance tests (table 2) were performed on five of our rabbits that survived longer than four days (see table 1), all of which failed to show glycosuria. A latent diabetic disturbance was found to exist in two (*nos. 14* and *19*) and total absence of any diabetic disturbance was observed in three (*nos. 1, 5* and *21*).

TABLE 2. DEXTROSE TOLERANCE TESTS IN FIVE RABBITS THAT SURVIVED LONGER THAN FOUR DAYS AFTER THE EPINEPHRINE ALLOXAN TREATMENT AND SHOWED NO GLYCOSURIA LATER THAN 24 HOURS AFTER THE ALLOXAN INJECTION

RABBIT NO.	BLOOD SUGAR 24 HOURS AFTER ALLOXAN INJECTION	HIGHEST FASTING BLOOD SUGAR LATER THAN 24 HOURS	SUGAR TOLERANCE TEST			RESULTS
			Day after epinephrine-alloxan injections	Blood sugar		
				Fasting	2 hours	
	mgm/100 cc.	*mgm/100 cc.*		*mgm/100 cc.*	*mgm/100 cc.*	
1	77		12th	126	117	No diabetes[1]
5	117	100	6th	93	93	No diabetes[1]
14	410		8th	38	155	Latent diabetes
19	124	124	6th	124	320	Latent diabetes
21	171	132	5th	109	95	No diabetes[1]

[1] A few days after the dextrose tolerance test had shown absence of any diabetic disturbance, *rabbits no. 1, 5* and *21* were again injected with alloxan, without previous epinephrine injection, and all three developed typical diabetes.

In these three rabbits alloxan was again given a few days later, without a previous epinephrine injection, and all three developed typical diabetes. This proved that the previous failure of these animals to become diabetic was not due to an individual lack of sensitivity to alloxan. The same was shown, in quite a different way (table 1), for two of these rabbits (*nos. 1* and *5*), as well as for others that failed to show any evidence of typical diabetes (*nos. 4, 6, 8, 19* and *20*), by the fact that all these animals showed alloxan hypoglycemia.

At least in two epinephrine alloxan-treated rabbits that died from alloxan hypoglycemia (*nos. 11* and *12*, table 3), the pancreatic islets, as well as the rest of the pancreas, were found to be histologically normal. From the presented facts the statement seems justified that, under the conditions of our experiments, intramuscular injection of epinephrine in doses of 200 micrograms per kgm. of body weight before the administration of commonly used diabetogenic doses of alloxan, in most cases, either very markedly hindered or totally abolished the diabetogenic effect of alloxan in rabbits.

The above results appear to be independent of sex, age or weight of the rabbits used and of fasting or nonfasting prior to the experiment.

The fact that all the rabbits that were protected by epinephrine against the diabetogenic action of alloxan, and that were not given dextrose injections early after the alloxan treatment, showed hypoglycemia suggests that alloxan was not chemically inactivated by epinephrine. Furthermore, the variability of the antidiabetic effect of epinephrine under similar experimental conditions is also more in accordance with a biological than with a simple chemical mechanism. The biological mechanism that protects the pancreas against the diabetogenic action of alloxan may well be the vasoconstriction which is known to be elicited by epinephrine on the pancreatic vascular system (5–8) in the rabbit. It should be pointed out that in the case of *rabbits no. 13* and *14* less than 20 minutes elapsed between the epinephrine and the alloxan injections as compared to 60 to 80 minutes with all other rabbits of our series. These 2 animals were among 3 (*no. 9*, *13* and *14*) which, out of 11 that survived more than 24 hours, showed unmistakable diabetic hyperglycemia. Whether this is mere coincidence or whether an interval longer than 20 minutes between epinephrine and alloxan injections is necessary to prevent the diabetogenic action of the latter can only be decided by further research.

TABLE 3. DATA CONCERNING 11 RABBITS (OUT OF A SERIES OF 22) THAT DIED WITHIN 24 HOURS AFTER AN INTRAVENOUS INJECTION OF A CURRENT DIABETOGENIC DOSE OF ALLOXAN PRECEDED BY AN INTRAMUSCULAR INJECTION OF A DOSE OF 200 MICROGRAMS PER KGM. OF EPINEHPRINE

RABBIT NO.	WEIGHT	ALLOXAN PER KGM.	PRELIMINARY FAST	TIME AFTER ALLOXAN WHEN CONVULSIONS APPEARED FIRST	TIME AFTER ALLOXAN WHEN DEXTROSE INJECTIONS WERE GIVEN		TIME OF DEATH (AFTER ALLOXAN)	CAUSE OF DEATH
					First	Last		
	grams	*mgm.*	*hours*	*hours*	*hours*	*hours*	*hours*	
2	1925	185	24	2	2	7	more than 16	Hypoglycemia ?
3	1210	204	24	2¾	2¾		2¾	Hypoglycemia
7	1920	200	24	17	5½	12	more than 17	Hypoglycemia
10	950	200	24	4	4	12	more than 16	Hypoglycemia ?
11	1190	200	24	3¼	3¼		3¼	Hypoglycemia
12	1230	200	24	2	2		2	Hypoglycemia
15	1250	200	24	2¼	2¼	6	6	Hypoglycemia
16	1390	200	24	2	2	6	6	Hypoglycemia
17	1320	200	21		2	12	12½	Pulmonary edema
18	1185	200	21		2		3	Pulmonary edema
22	1415	180	none	3¼	3¼		3¼	Hypoglycemia

Fifty per cent of our rabbits died within 24 hours (table 3) and in most of these animals the cause of death was, in all probability, hypoglycemia. Four rabbits, *no. 3*, *11*, *12* and *22* died within three hours and fifteen minutes after the alloxan injection and quite suddenly after the appearance of hypoglycemic convulsions, leaving no opportunity for effective treatment; two other rabbits, *no. 15* and *16* died six hours after the injection of alloxan in hypoglycemic convulsions, despite previous and repeated dextrose injections; two others died from pulmonary edema. The other three animals that died within 24 hours did so late in the night, 16 hours or longer after the alloxan injection. They had received several dextrose injections and the watch had been discontinued, since, in the light of previous experience from our own and other laboratories (2, 3, 4), it seemed that there should be no more danger of death from hypoglycemia.

Nearly all of our animals that died within 24 hours had been fasted for 21 to 24 hours and most of them were very young. We cannot rule out the possibility that these factors might have something to do with the high mortality from hypoglycemia, its early appearance and its late recurrence in our experiments. With this reservation we feel that the features of the hypoglycemia shown by these animals were a result of epinephrine-alloxan treatment as detailed above.

Low, and even very low, blood sugar figures were recorded in several animals 24 hours or more after the alloxan injection (table 1) as follows: *no. 8*, 62 at 24 hours; *no. 20*, 65 at 24 hours and 50 at 48 hours; *no. 6*, 37 at 24 hours; and *no. 14* (table 2), 38 as late as on the eighth day. There can be no doubt that this extremely late persistence or recurrence of hypoglycemia is a result of the epinephrine-alloxan treatment, since nothing similar has been reported as a result of simple alloxan treatment in rabbits or other species.

In rabbits *no. 1* and *5*, the epinephrine-alloxan treatment did not produce diabetes (table 2) but did produce alloxan hypoglycemia (table 1). This observation, which is the first of its kind to be reported, conflicts with Goldner and Gomori's statement (9) that alloxan hypoglycemia has never been observed in animals that survived and failed to become diabetic, and with the widely accepted view that alloxan hypoglycemia is due to insulin released from the islets of Langerhans in the course of their destruction under alloxan action. This view is also opposed by the fact that in our rabbits, *no. 11* and *12*, that died from alloxan hypoglycemia (table 3), the pancreas was found to be histologically normal. From the point of view of the mechanism of production of alloxan hypoglycemia, our results bear out Houssay's contention (10) that alloxan hypoglycemia is not due to insulin released from the damaged pancreas but is brought about by some extra pancreatic mechanism, probably as a result of alloxan injury to the liver.

Finally, out of 11 animals that survived 24 hours or longer, six (*no. 4*, *6*, *8*, *13*, *14*, and *20*, table 1) died within 2 to 8 days. In all probability, the cause of death of these animals was not diabetes but liver and/or kidney damage. This means an exceptionally high mortality as a result of alloxan-induced damage other than diabetes. Therefore, while realizing that further study is required to reach any definite conclusion on this matter, we feel that a previous injection of epinephrine, under the particular conditions of the reported experiments, tends to increase the damage induced by alloxan in organs or tissues other than the pancreas and, as a result, tends to increase nondiabetic alloxan mortality.

SUMMARY

Intramuscular injection of epinephrine in doses of 200 micrograms per kgm. of body weight given 60 to 80 minutes before administration of diabetogenic doses of alloxan to rabbits either very markedly hindered or totally abolished the diabetogenic effect of alloxan in most cases. The mechanisms of this phenomenon and the bearing of some experimental factors upon it are briefly discussed.

The results of our experiments suggest that a previous injection of epinephrine tends to alter the effects of alloxan by favoring an earlier appearance, a greater severity and a later recurrence of hypoglycemia, and by increasing the incidence and the severity of, as well as the mortality from, alloxan-induced damage other than diabetes.

We wish to thank Dr. R. Barroso, to whom we are indebted for the histological study of the pancreas and other organs of our rabbits and Dr. S. Zubirán for his kind interest in our work.

REFERENCES

1. Kass, E. H. and B. A. Weisbren. Proc. Soc. Exp. Biol. Med. **60:** 303, 1945.
2. Bailey, O. T., C. C. Bailey and W. H. Hagan. Am. J. Med. Sci., **208:** 450, 1944.
3. Rojas, E., La Diabetes Aloxánica en el Conejo. Thesis for M.D. degree, University of México, 1945.
4. Zubirán, S. Rev. Med. Hospital General México, **8:** no. 8, 1946.
5. Edmunds, C. W. J. Pharmacol. Exper. Therap. **1:** 135, 1909–1910.
6. Mann, J. C. and L. C. McLachlen. J. Pharmacol. Exp. Therap. **10:** 251, 1917.
7. May, O. J. Physiol. **30:** 400, 1904.
8. Natus, M. Virchow's Arch. **199:** 1, 1910.
9. Goldner, M. G. and G. Gomori. Proc. Am. Diab. Assoc. **4:** 89, 1944.
10. Houssay, B. A., O. Orias and J. C. Sara. Rev. Soc. Argentina Biol. **21:** 30, 1945.

EXCRETION OF URINARY 17-KETOSTEROIDS BY THE MALE RABBIT

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Zimmerman (1, 2) observed that ketosteroids react with *m*-dinitrobenzene in an alkaline medium to produce a red color. Subsequently, it was found that in diluted solutions the optical density of the color is proportional to the concentration of the 17-ketosteroids within rigid conditions of assay. The Zimmerman reaction has been applied with considerable success to assay the 17-ketosteroid content of human urine extracts. According to the available evidence the urinary 17-ketosteroids are metabolites derived from the adrenal cortex and testes (3, 4). There have been few reports in which the Zimmerman reaction has been applied to the urine extracts of experimental animals. A Zimmerman reaction applied to urine extracts of rats is included in a report by Lampton and Miller (5). Crude neutral urine fractions were assayed using an aqueous modification of the Zimmerman colorimetric reaction. Karnofsky, Nathanson, and Aub (6) have reported the 17-ketosteroid excretion in the mouse. From their report it appears that crude neutral fractions were used. Unfortunately, neither report present data to substantiate the methods used in their studies.

Since the nature and quantity of the urinary chromogens are unknown it was deemed important to test the applicability of the assay method for urine analysis of an experimental animal. The quantity of urinary 17-ketosteroids excreted by the male rabbit was studied, and data concerning the extent of correlation between volume and 17-ketosteroid excretion analyzed. Also, pathologies were induced in the adrenal glands and changes in the excretion of 17-ketosteroids determined.

MATERIALS AND METHODS

Eighteen mature male rabbits weighing 9.5–11.5 pounds were used. The colony was maintained under optimum conditions for at least six weeks prior to collection and assay of urine samples. They were fed complete rabbit pellets, and water was supplied in heavy stone crocks to avoid dilution of urine collections.

A quarter-inch mesh screen was placed beneath individual wire rabbit cages which sifted the fecal pellets but allowed the urine to pass through the mesh. Urine drained down a *V*-shaped tray tilted into a collection flask containing 4 cc. of concentrated HCl for preservation of the sample. Accurately timed 48-hour urine specimens were collected. Assays were made with 250 ml. aliquots of the total 48-hour collection. If the total urine collection was less than 250 ml. it was diluted to 250 ml. with distilled water. Urines were filtered and stored in the refrigerator until preliminary extraction which was completed within three days of the collection periods.

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The crude neutral fraction. Urines were hydrolized by refluxing with 15 per cent by volume of concentrated HCl for seven minutes under 'cold finger' condensers. The hydrolized urine was cooled and immediately extracted four times with 35 ml. aliquots of ethyl ether. Combined ether extracts were washed once with water and twice with saturated Na_2CO_3 solution. Phenolic compounds present in the ether were removed by three washings with 2N·NaOH. Subsequently, the ether extract was washed three times with distilled water to remove alkalies. The ether solvent was then distilled off and the crude neutral urinary extracts dried and stored *in vacuo*.

Ketonic fractionation. The crude neutral fraction further treated with 40 mgm. of Girard's Reagent *T* according to the micro-method of Pincus (7) and a partially purified ketonic fraction obtained.

TABLE 1. 17-KETOSTEROIDS (MGM/250 ML.) DETERMINED FOR ALIQUOTS OF POOLED URINE AND COMPARED FOR PRECISION OF TECHNIQUE

ALIQUOT	POOL NO.							
	1	2	3	4	5	6	7	8
#1	5.8	6.5	5.8	2.5	5.2	1.3	5.5	6.2
#2	5.7	6.3	5.9	1.7	5.2	1.4	5.6	6.0
#3	6.1	6.7	6.2	2.0	5.1	0.9	5.2	5.9
#4	6.0	6.5	6.1	—	—	—	—	—
Average	5.9	6.5	6.0	2.1	5.2	1.2	5.4	6.0

Assay for 17-ketosteroids. An anhydrous modification of the Zimmerman colorimetric reaction developed by Talbot *et al.* (8) was used to assay the samples. For assay the urinary ketonic fraction was dissolved in 5.00 ml. of absolute ethanol. The optical density of the colorimetric reaction was measured in an Evelyn photoelectric colorimeter with a Rubicon filter having maximum transmission of 520 m.μ.

In order to evaluate the galvanometer readings of the urinary ketonic fractions the colorimeter was previously calibrated with six known concentrations of crystalline dehydroisoandrosterone[2] over a range of 50–300 γ. With each series of urine assays a standard was also assayed as a check on the technique. Each ketonic fraction, reagent blank, and standard was assayed in triplicate and the average of the three assays used as the representative value.

ANALYSIS OF THE METHOD

Overall precision for the technique was estimated from collections of rabbit urine pooled so that several 250 ml. aliquots were obtainable. The precision for comparable aliquots within eight such urine pools is presented in table 1.

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[2] Crystalline dehydroisoandrosterone was kindly supplied to the investigator by Dr. E. Schwenk, Schering Corporation.

Accuracy of the method is reflected in the recovery efficiency of crystalline 17-ketosteroids added to urine. In such experiments 12 aliquots of pooled urine were assayed while 11 other aliquots of the same urine pool received measured amounts (5.00–6.00 mgm.) of crystalline androsterone. The average recovery was 88 per cent of the androsterone added to the urine.

The reagents were studied in several ways to examine their non-specific chromogen contribution. As a check on the constancy of the blank color with different batches of reagents it was routine to adjust the galvanometer to 100° and obtain a setting with the reagent blank. The blank setting during 10 months varied between 78°–82°. A scan was made of the colorimetric reagent blank absorption compared with an absolute ethanol blank in a Beckman spectrophotometer over a range of 400–640 m.μ. The resultant curve revealed maximum absorption at 450 m.μ. which rapidly decreased to a low level at higher wave lengths. Along with several urine samples a 250 ml. aliquot of distilled water was processed as if it were a routine urine collection. The water sample showed no detectable assay value. Hence, it was assumed that the reagents used did not contribute chromogen which interfered with the assay method.

TABLE 2. COMPARISON OF ASSAY VALUES FOR CRUDE NEUTRAL EXTRACTS AND KETONIC FRACTIONS (MGM/250 ML.) OBTAINED FROM ALIQUOTS OF POOLED URINE

URINE POOL	CRUDE FRACTION	KETONIC FRACTION	% CHROMOGEN AS KETONIC FRACTION
I	2.9	1.6	54.0
II	3.0	1.4	47.5
III	4.1	2.1	50.9
IV	4.0	2.1	54.0
V	3.0	1.6	53.7

According to test experiments the technique of ketonic fractionation resulted in the largest loss of 17-ketosteroids. To determine the value of such purification in rabbit urine several aliquots of pooled urine were assayed in the crude neutral state while other aliquots of the same pool were purified to the ketonic stage. The assay values of the two fractions are compared in table 2. The average reduction in chromogen content is 52 per cent.

Optical density curves for the colorimetric reaction of various urine fractions and crystalline dehydroisoandrosterone were derived in a Beckman spectrophotometer at 20 m.μ. intervals from 400 to 640 m.μ. The crude neutral fraction of rabbit urine shows maximum density near 420 m.μ. and a second peak at 520 m.μ. The ketonic fraction has a single peak at 520 m.μ. and resembles the bell-shaped curve of crystalline dehydroisoandrosterone with the exception of relatively high density at 420 m.μ. The non-ketonic fraction has a peak at 420 m.μ and rapidly falls off in density at higher wave lengths. Such curves are roughly comparable to those obtained from the same fractions of human urine (9).

To determine the extent of non-specific chromogen the ratio of extinction coefficients $E_{520}:E_{420}$ was recorded for the fractions of rabbit urine. These ratios reveal that interfering chromogen is reduced by obtaining the partially purified ketones. A comparison of assay values with their respective extinction coefficient ratios suggests that as the concentration of 17-ketosteroids decreases the interference due to non-specific chromogen increases.

TABLE 3. AVERAGE 17-KETOSTEROID AND URINE EXCRETION PER 48 HOURS OF 18 NORMAL ADULT MALE RABBITS

ANIMAL	NO. OF URINES	MGM. 17-KS	S.E. $\pm\sigma_m$	ML. URINE
A	9	2.24	0.19	402
B	10	2.27	0.22	347
C	10	2.21	0.20	249
D	10	2.57	0.20	327
E	9	2.25	0.22	280
F	10	3.45	0.30	311
G	10	2.45	0.22	247
H	3	2.56	0.27	852
I	6	1.55	0.08	318
J	4	2.50	0.05	459
K	4	2.76	0.34	467
L	4	2.01	0.19	270
M	3	1.88	0.38	422
N	3	2.67	0.48	1080
O	3	1.91	0.23	283
R	4	1.78	0.05	284
S	4	1.28	0.15	343
T	4	1.93	0.12	287

TABLE 4. CONTINUOUS EXCRETION OF 17-KETOSTEROIDS (MGM/48 HRS.) ASSAYED FOR SEVEN NORMAL MALE RABBITS (FIGURES IN PARENTHESES ARE THE MEAN VALUES)

RABBIT A	RABBIT B	RABBIT C	RABBIT D	RABBIT E	RABBIT F	RABBIT G
2.03	1.50	2.50	2.69	1.72	1.96	2.30
2.76	3.32	3.17	3.91	1.35	2.58	2.31
1.72	1.42	1.20	2.41	1.69	3.14	1.81
2.48	2.12	1.85	2.33	2.08	3.24	3.42
2.28	2.45	2.52	2.01	2.88	4.79	2.81
2.14	2.42	2.53	1.82	1.95	5.14	1.65
—	2.72	1.35	3.01	—	4.35	2.27
3.38	3.49	2.90	3.28	3.44	3.44	3.96
2.11	1.71	2.37	2.35	2.76	2.90	2.15
1.31	1.56	1.69	1.85	2.51	2.95	1.80
(2.24)	(2.27)	(2.21)	(2.57)	(2.25)	(3.45)	(2.45)

APPLICATION OF THE METHOD

In order to determine the excretion of 17-ketosteroids in normal adult male rabbits, 109 collections of 48-hour urine samples from 18 animals were assayed.

Table 3 summarizes the average 17-ketosteroid excretion for each animal of the colony. Individual rabbits vary in average excretion of 17-ketosteroids from 1.28 to 3.45 with the average excretion of the colony as 2.23 mgm/48 hours. Standard deviation of variates was ±0.48 or 22 per cent of the mean value. Among 109 urine collections assayed the excretion of total 17-ketosteroids varied from 0.86 to 5.14 with a mean sample value of 2.31 mgm. per 48 hours and a standard deviation of ±0.79 which is 34 per cent of the mean.

TABLE 5. AVERAGE 17-KETOSTEROID EXCRETION (MGM/48 HRS.) OF RABBITS IN NORMAL AND EXPERIMENTAL PATHOLOGIES INDUCED IN THE ADRENAL GLANDS

ANIMAL	NORMAL LEVEL	ME-CHOL. 305 DAYS	PERCENT DECREASE
D	2.57	1.95	25
E	2.25	1.59	30
F	3.45	1.62	50
G	2.45	2.28	7
		B-P TUMOR INVOLVEMENT	
W	2.31[1]	0.33	86
X	2.31[1]	0.18	92

[1] Excretion level of the colony.

TABLE 6. ANALYSIS OF COVARIANCE[1] OF URINE VOLUME (X = ML/48 HRS.) AND 17-KETOSTEROIDS (Y = MGM/48 HRS.) FOR SEVEN NORMAL RABBITS STUDIED FOR 9 OR 10 CONSECUTIVE COLLECTIONS OF 48 HOURS' DURATION

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES AND PRODUCTS			ERRORS OF ESTIMATE	
		Sx^2	Sxy	Sy^2	Sums of squares	Degrees freedom
Among rabbits	6	177237	6.68	4.5234		
Within rabbits	61	310217	2123.30	40.7034	26.1703	60
Total	67	487454	2129.98	45.2268	35.9196	66
Adjusted among rabbits					9.7493	6

[1] See, for example, *Statistical Methods*, G. W. Snedecor, Iowa State Press, 1940.

To determine the extent to which 17-ketosteroid excretion varies in individual normal animals the continuous urinary excretions of seven animals were studied for 20 days. Table 4 lists the excretions of these animals.

In an endeavor to alter the rate of adrenal function certain pathologies were induced which involved the adrenal cortical gland. Two black Dutch rabbits received inoculations of Brown-Pearce carcinoma suspension into the testes. Urine collections were begun seven days after inoculation. *Animal X* collapsed on the eleventh day so that two complete 48-hour urine collections were obtained. Three 48-hour collections were obtained from *animal W* before this animal succumbed. Although the collections were of normal urine volumes the 17-ketosteroid output was less than 0.5 mgm/48 hours in all samples (table 6).

Histologic sections of the adrenal glands exhibited numerous large metastases of Brown-Pearce tumor cells. The rapidly proliferating carcinoma appeared to have replaced and crowded out the normal adrenal cells.

Another group of animals (*D*, *E*, *F*, *G*), whose normal 17-ketosteroid excretion level was established, were treated with 20-methyl-cholanthrene suspended in olive oil. Adrenals of each animal were exposed surgically, and 3–5 mgm. of methyl-cholanthrene deposited in each adrenal. Three hundred days later urine samples were collected continuously for eight days and assayed.

The level of 17-ketosteroid excretion was depressed in *animals D*, *E*, and *F*, while *animal G* excreted 17-ketosteroids at its pretreatment level (table 6). Histologic examination of adrenal sections revealed two unique cell types. Present in the adrenals of *animals D*, *E*, and *F* were many thin cords of very small intensely chromatic cells. These dark cell cords were very prominent immediately beneath the capsule of the cortex. They could not be identified as usual morphological constituents of the adrenal cortex. The second unusual cell type were clusters of highly vacuolated cells. These 'foam' cells were rare and found only in glands of *animals E* and *F*.

DISCUSSION

On the basis of data reported here concerning the preparation of rabbit urine extracts and their subsequent assay, the method has been demonstrated as being sufficiently quantitative, precise, and accurate enough to warrant application in animal studies.

The absorption spectra of various urine fractions indicate the ketonic fraction most closely resembles the curve of crystalline 17-ketosteroids, while fractionation experiments indicate that about half of the chromogen present in the crude neutral fraction is inert (table 2). In order to obtain extracts of sufficient chromogen purity it appears essential to obtain ketonic fractions. The excretion level of 17-ketosteroids in normal rabbits shows approximately the same purity of chromogen as the low level human urines reported by Talbot *et al.* (11).

It has been demonstrated that the principal 17-ketosteroids present in urine develop almost identical chromogen density in the relatively anhydrous assay modifications of the Zimmerman reaction (9, 10) but not with techniques using water as a reagent solvent. Since the constituent 17-ketosteroids of rabbit urine have not been characterized chemically, it appears advisable to use an anhydrous assay technique despite greater difficulty in preparation of alcoholic reagents.

To examine the relationship of urine volume to 17-ketosteroid excretion of rabbits an analysis of covariance was computed[3] (table 6). The analysis was based upon a study of 48-hour samples collected continuously for 18 or 20 days on seven normal animals. There were 68 samples analysed for the correlation of urine volume with steroid content.

The data show statistically significant differences among the average urine volumes of the individual rabbits ($F = 5.7$[4] where $F_{.05} = 2.25$) while the differences among average 17-ketosteroid excretions for the seven individuals are not significant ($F = 1.13$ where $F_{.05} = 2.25$). Average correlation of the urine output to the 17-ketosteroid excretion on the basis of the seven individual animals is 0.60 and the correlation based on all the 68 urine collections is 0.45. The average values for urine volume and 17-ketosteroid excretion for the seven rabbits are not correlated. From such information it is obvious that excretion of 17-ketosteroids is relatively constant despite the variations in urine volumes.

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[3] The analysis of covariance was performed by the University of Oregon Computation Service under the direction of Dr. W. J. Dixon, Department of Mathematics, University of Oregon.

To estimate the value of urine volume for predicting the 17-ketosteroid content a percentage comparison was made of the actual variance of y (mgm. 17-ketosteroid/48 hours) with the variance of y predicted on the basis of x (ml. urine/48 hours). The 'percent of information' or 'efficiency' of urine volume as an estimate of 17-ketosteroid content is $0.4362/0.6673 = 65$ per cent.[5] The 17-ketosteroid excretion is considerably more constant when determined as a function of time duration rather than urine volume.

It is not known whether the depressed levels of animals receiving the Brown-Pearce carcinoma are due to a general debility of these animals near the height of metastases, or whether the resultant levels are directly attributable to the replacement and crowding out of active adrenal cells by the large metastases of tumor cells. The latter view is most adequately supported by histological studies of the adrenal sections.

The histological changes induced in the adrenals after 300 days exposure to methyl-cholanthrene do not appear to be typical adenoma of cortical cells but rather a proliferation of an anaplastic, densely chromatic type of cell. Since there were no other pathologies in these animals the depressed excretion of 17-ketosteroids is related to the changes in the adrenal glands. The experimentally induced pathologies of the adrenal gland suggests that interference with normal adrenal function lowers the excretion of 17-ketosteroids.

SUMMARY

An anhydrous Zimmerman reaction was applied to a partially purified neutral ketonic fraction of rabbit urine. The preparation of the fraction and its subsequent assay for 17-ketosteroids was studied to determine the applicability of the method used. The data of precision studies, recovery experiments, and studies of the chromogen purity indicate that the method is sufficiently accurate to warrant application utilizing the rabbit. In order to obtain fractions of sufficient chromogen purity it is essential to use ketonic fractions since the crude neutral fraction contains excessive chromogenic materials which yield false steroid titer.

The average level of 17-ketosteroid excretion in the normal male rabbit is 2.23 mgm. per 48 hours as indicated by assay of 109 urines collected from 18 animals.

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[4] $F = \dfrac{177237/6}{310217/61} = 5.7$ (table 6).

[5] $26.1903/60 = 0.4362$ and $40.7034/61 = 0.6673$ (table 6).

A covariance study of the urine volumes excreted reveals that the excretion of 17-ketosteroids is relatively constant despite variations in urine volume.

Inoculation of Brown-Pearce tumor suspension into two animals reduced the excretion of 17-ketosteroids to less than 0.5 mgm. per 48 hours. The adrenal glands were invaded by large metastases of tumor cells. Methyl-cholanthrene deposited in the adrenal glands of four animals for 300 days induced histologic changes in the adrenal cortical gland. Three of these animals excreted a significantly lower level of 17-ketosteroids.

Depression of 17-ketosteroid excretion is correlated with changes in adrenal function as evidenced by the histology of the gland. Such evidence supports the adrenal source hypothesis of 17-ketosteroids.

The author wishes to thank Dr. Boris Krichesky for suggestions and criticisms made during the course of the study. I am grateful to Dr. Elly Jacobsen for the Brown-Pearce injected animals.

REFERENCES

1. Zimmerman, H. Ztschr. f. Physiol. Chem. **233:** 257, 1935.
2. Zimmerman, H. Ztschr. f. Physiol. Chem. **245:** 47, 1936.
3. Fraser, R. W., A. P. Forbes, F. Albright, H. Sulkowitch, and E. Reifenstein. J. Clin. Endocrinology **1:** 234, 1941.
4. Pincus, G. J. Clin. Endocrinology **3:** 655, 1943.
5. Lampton, A. K. and A. J. Miller. J. Urology **45:** 552, 1941.
6. Karnofsky, D., I. Nathanson, and J. Aub. Canc. Res. **4:** 772, 1944.
7. Pincus, G. J. Clin. Endocrinology **5:** 291, 1945.
8. Talbot, N. B., A. Butler, and E. A. MacLachlan. J. Biol. Chem. **132:** 595, 1940.
9. Wilson, H. and I. T. Nathanson. Endocrinology **37:** 208, 1945.
10. Saier, M., R. Warga and R. Graves. J. Biol. Chem. **137:** 317, 1941.
11. Talbot, N. B., R. A. Berman and E. A. MacLachlan. J. Biol. Chem. **143:** 211, 1942

INHIBITION OF EPINEPHRINE ACTION IN SEVERE HYPOXEMIA[1]

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Recently we have had occasion to observe the changes in blood pressure and heart rate in dogs in which the oxygen tension of the blood was reduced rapidly from normal values to a nearly absolute anoxemia. This acute form of severe hypoxemia was produced by 100 per cent nitrogen breathing. Under these conditions, the blood pressure may rise for a short period, as has been noted by several investigators (1–4). However, as the hypoxemia continues, the blood pressure falls progressively to low levels. Restoration of oxygen breathing causes a marked rise in blood pressure, considerably above the hypoxemia level.

We have found that epinephrine injected during the hypoxemic depressor phase fails to give the usual pressor response. Instead a reduced or absent response is seen as long as the hypoxemia continues. When oxygen is again introduced into the breathing mixture, the epinephrine then produces its pressor effect. This epinephrine action suggested a failure of responsiveness of the heart and blood vessels during extreme hypoxemia and indicated that the epinephrine could not be metabolized in the absence of oxygen, since a marked pressor effect appeared when the animal again breathed air. The lack of response to a potent agent such as epinephrine and its unexpected latency of action in severe hypoxemia led us to investigate the mechanisms involved. In this communication we report our results 1) on the blood pressure and heart rate changes following the induction of almost complete hypoxemia and after the subsequent reinstitution of air breathing and 2) on the response to the injection of epinephrine before, during and after the hypoxemic phase.

METHODS

Thirty-four dogs weighing from 6 to 17 kgm. were anesthetized with intravenous pentobarbital sodium (35 mgm/kgm.). Tracheotomy was performed and the vagi sectioned. Blood pressures were recorded from the femoral artery with a mercury manometer via ink writing on kymograph paper, or with the Hamilton manometer with optical recording of the pressure pulses. In 32 animals, the chest was opened by splitting of the sternum, or by incision in the fifth or sixth intercostal spaces with wide retraction of the ribs and removal of the mediastinal septum. Positive blast respiration was used.

It was found necessary to open the thorax to induce hypoxemia rapidly. Thus, when the positive pressure air respirator was disconnected from the tracheal cannula, the lungs collapsed and expelled most of their gaseous contents. Within

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[1] Aided by the A. D. Nast Fund for Cardiovascular Research. The Department is supported in part by the Michael Reese Research Foundation.

a second or two, the tracheal tube was connected to positive blast from a nitrogen cylinder. On inflation with the nitrogen, the partial pressures of the gases in the lungs were probably now partitioned approximately as follows: a nitrogen tension of nearly 650 mm. Hg, a water vapor tension of about 50 mm. Hg (saturation at body temperature), an oxygen tension approaching zero, and the remainder carbon dioxide. Under these conditions, the circulating blood reaching the pulmonary capillaries quickly lost its oxygen via the lungs. Carbon dioxide was lost as in the normal animal. In this way we were able to set up a high oxygen gradient from the tissues to the lungs without accumulation of carbon dioxide and rapidly deplete the oxygen reserves of the animal. As indicated by the blood pressure response this rapid rate of deoxygenation did not obtain when the chest was not opened since the lungs did not collapse, and some oxygen remained in the alveoli. Also, by giving artificial respiration we were able to eliminate the effects of respiratory failure which occurs commonly before the failure of the circulation.

TABLE 1. TIME AND LEVEL OF THE MAXIMAL HYPOXEMIC BLOOD PRESSURE RISE

DURATION OF HYPOXEMIA	NUMBER OF TRIALS WITH HYPOXEMIC RISE	AVERAGE MAXIMAL RISE OBTAINED FOR EACH GROUP
seconds		*mm. Hg*
15	0	—
30	6	21
45	9	29
60	16	33
75	9	37
90	11	25
105	8	51
120	1	46
Total....................	60	

At the end of periods of nitrogen breathing up to 180 seconds, the respirator tube was disconnected from the nitrogen tank and, after a second or two, reconnected to the air supply. This reversal of oxygen gradient caused a rapid return of the blood oxygen saturation to normal levels.

In some experiments epinephrine (0.5 mgm.) was injected into the femoral vein or into the left ventricle at various times, before and during the hypoxemic phase and also during the post-hypoxemic re-oxygenation phase.

RESULTS

1. *Effect of hypoxemia on the blood pressure.* Immediately after the institution of nitrogen breathing, in 60 out of 70 trials, a progressive rise in mean arterial pressure for periods varying from 30 to 120 seconds and averaging 70 seconds (hypoxemic pressor phase) was observed. The average rise observed was 33 mm. Hg, but in some instances it rose as much as 120 mm. Hg. The average data are given in table 1, and typical records in figures 1 and 4. No hypoxemic rise

was seen in 10 trials in 2 dogs which consistently failed to show this hypoxemic rise.

As the hypoxemia continued, the blood pressure leveled off, was maintained at its peak value for 15 to 45 seconds and then began to fall abruptly (hypoxemic depressor phase). This occurred usually after about 75 seconds of hypoxemia (range 30 to 120 seconds). Within 30 seconds, most of the blood pressures had already fallen to the pre-hypoxemic level. The pressure continued to fall to very low levels with circulatory failure and extreme dilatation of the heart occurring within a few minutes.

2. *Effect of re-oxygenation.* After 0 to 90 seconds in the hypoxemic depressor phase, air breathing was reinstituted in 65 trials. In 56 of these (90 per cent) the blood pressure was seen to rise sharply within the first 10 seconds. In most instances this pressor action produced values considerably above the control blood pressure level and even above the maximal pressure attained in the hypoxemic pressor phase. The time of occurrence of the maximal pressure levels and their increase above the control level are shown in table 2.

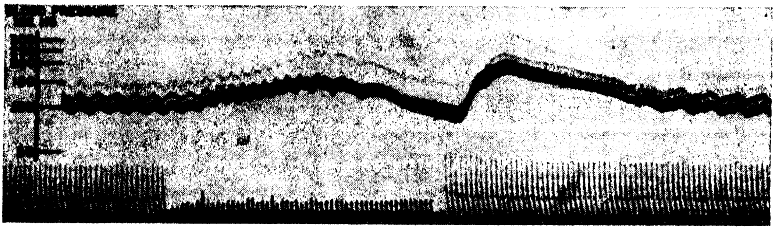


FIG. 1. EFFECT OF BREATHING 100 per cent nitrogen and reinstitution of air breathing in open-chested anesthetized dogs on the blood pressure recorded optically with the Hamilton manometer. Upper curve is blood pressure; lower curve, positive pressure respiration via automatic respirator (upstroke represents inspiration). Nitrogen breathing begun at break in respiration record at which amplitude of respiration line decreased. Air breathing resumed at break at which amplitude increased. Time, in seconds, recorded at bottom. Note the pressor effect early in hypoxemia, later giving way to a progressive fall in pressure. On resumption of air breathing, a marked pressor effect is seen.

3. *Relation between the duration of the hypoxemic depressor phase and the degree of the post-hypoxemic blood pressure rise.* A correspondence was found between the duration of the hypoxemic depressor phase and the degree of the post-hypoxemic rise. This is illustrated in figure 2 on data from three consecutive experiments. It can be seen from the curve that the post-hypoxemic pressor effect was minimal in animals subjected to short periods of hypoxemia (60 secs.) and in which no significant depressor phase was noted. In the animals in which a depressor phase was seen, i.e., those with a longer period of hypoxemia, the post-hypoxemic pressor effect was noticeably increased, and roughly in proportion to the duration of the depressor phase.

The duration of the post-hypoxemic pressor phase, measured as the time of return from the peak attained during the post-hypoxemic rise to the control pressure level, was in general a function of the magnitude of the rise above the previous level.

TABLE 2. TIME OF OCCURRENCE OF THE MAXIMAL PRESSURE LEVELS DURING THE POST-HYPOXEMIC PRESSURE PHASE

DURATION OF THE POST-HYPOXEMIC PERIOD	NUMBER OF TRIALS WITH MAXIMAL RISE ATTAINED IN EACH STATED TIME PERIOD	AVERAGE MAXIMAL RISE ABOVE CONTROL VALUES OBTAINED FOR EACH GROUP
seconds		*mm. Hg*
15	5	44
30	14	76
45	16	68
60	8	77
75	5	59
90	2	87
105	2	38
120	2	27

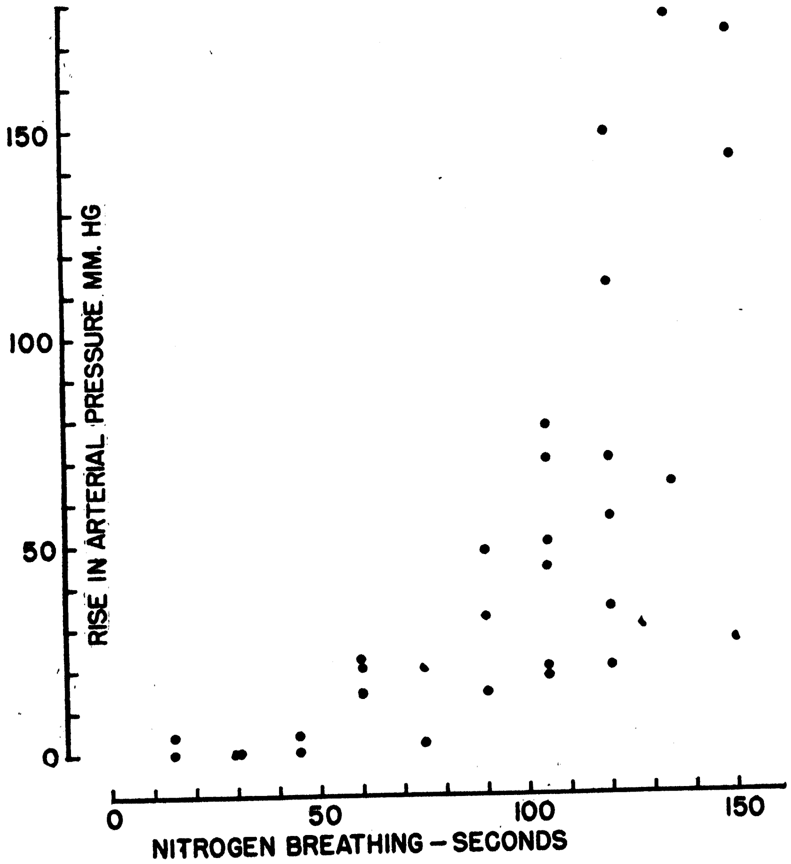


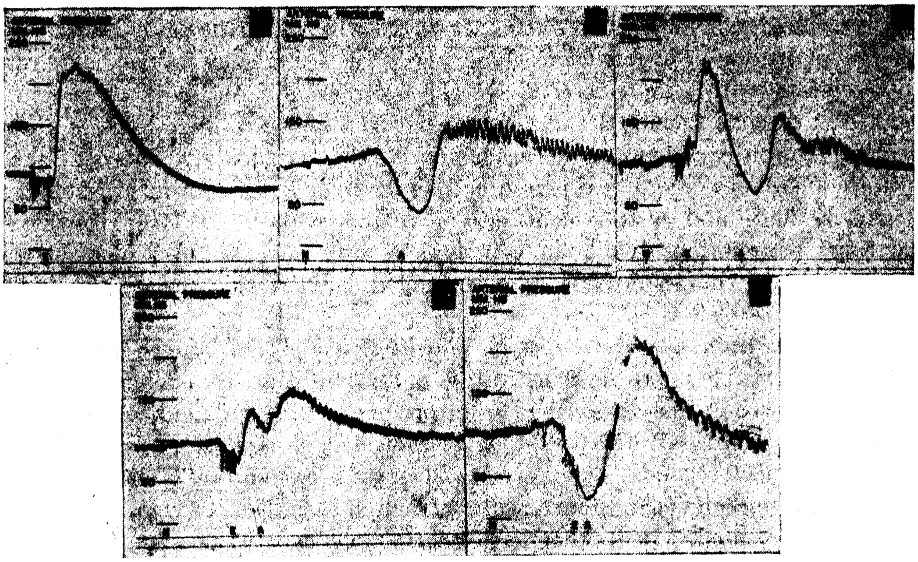
FIG. 2. SCATTERGRAM RELATING DURATION OF HYPOXEMIA to the post-hypoxemic rise in arterial pressure. Abscissae give time in seconds of nitrogen breathing; ordinates, rise in blood pressure above control level in post-hypoxemic period.

4. *Cardiac changes observed during the administration of nitrogen.* Shortly after the institution of nitrogen breathing, there was a tendency for the heart rate to accelerate, with a more forceful systolic contraction. As the blood pressure peak was reached during the hypoxemia period, the heart was observed to be beating vigorously and at a somewhat faster rate than originally. This continued into the phase of falling blood pressure. As the hypoxemia continued, the heart began to dilate and systolic contraction became weaker. In late hypoxemia, the heart was enormously dilated, contractions were feeble and A-V block supervened. When the hypoxemia was continued beyond this phase, the heart stopped in diastole.

5. *Cardiac changes observed upon the reinstitution of air breathing.* The cardiac changes observable upon the reinstitution of air breathing were dependent upon the stage to which the anoxia had been prolonged. When the heart had previously become dilated, with weak contractions at a slowed rate, a latent period (usually of 15–30 seconds) supervened following the resumption of air breathing. The heart rate then began to increase, contraction became more vigorous and the heart size decreased. In another 15–30 seconds the heart was beating vigorously and rapidly. During this period, the blood pressure increased rapidly toward its post-hypoxemic peak. The greater the duration of hypoxemia and the more severe the cardiac depression before resumption of air breathing, the longer was the latent period before recovery was seen. This effect can be seen in table 2. In several instances the hypoxemic depression of the heart was so profound that recovery did not take place with reinstitution of air breathing. Manual massage of the heart sometimes aided in recovery in such instances, although occasionally ventricular fibrillation developed as a result of this procedure.

6. *Effect of hypoxemia on the blood pressure response to epinephrine.* The usual effect of an injection of 0.5 mgm. of epinephrine into the femoral vein or left ventricle of the normal dog is a very rapid rise in pressure averaging 166 mm. Hg, with dissipation of the pressor effect in four or five minutes (fig. 3). When epinephrine was given at the onset of the hypoxemic period, the usual pressor response was seen, but the rise was not sustained as the animal went into progressive hypoxemia (fig. 5). When the epinephrine was given during the period of nitrogen breathing just prior to the time when the maximal pressure level was expected, the blood pressure would begin to increase but would fall again after a few seconds; i.e., the epinephrine pressor effect appeared to be aborted. During the early hypoxemic depressor phase, epinephrine injection resulted in an evanescent rise in pressure which quickly gave way to a fall in pressure even more marked than occurred normally. When the epinephrine was given in the late hypoxemic depressor phase, no change in the downward slope of the pressure curve was seen (fig. 7).

Although the pressor effect of the injected epinephrine was inhibited during the hypoxemic phase, it was again manifested after the reinstitution of air breathing. This was seen in the increased vigor of cardiac systole and in a greater than normal arterial pressure rise in the post-hypoxemic phase (compare figs. 5, 6 and 7



FIGS. 3 TO 7. SUCCESSIVE EXPERIMENTS ON THE SAME ANIMAL

FIG. 3. EFFECT OF LEFT INTRAVENTRICULAR INJECTION of 0.5 mgm. epinephrine on the blood pressure (*upper curve*) when animal was breathing air. Pressure recorded with a damped mercury manometer from the femoral artery. Calibration at left. Bottom line indicates time in 15-second intervals. Injection at E (this gives rise to irregularities of pressure curve preceding injection due to artefacts introduced by inserting needle into left ventricle). Following E there is an immediate rise in pressure reaching a maximum in about 20 seconds and falling off during the next few minutes.

FIG. 4. EFFECT OF NITROGEN BREATHING (N) and reinstitution of air breathing (A) on the blood pressure. Conventions as in figure 3. There is a slight rise in pressure following nitrogen breathing giving way after about a minute to a rapidly falling pressure. Air breathing is followed after a brief latent period by a marked pressor response above the control and hypoxemic pressure levels. The pressure then falls slowly to the control level.

FIG. 5. EFFECT OF EPINEPHRINE INJECTED, as in figure 3, during the pressor phase of the nitrogen-breathing period. Conventions as in figures 3 and 4. Epinephrine was injected during pressor phase of hypoxemia. It caused a less marked and less sustained pressure effect than in figure 3; within about 30 seconds the pressure rise was replaced by an abrupt fall in pressure, steeper than in the depressor phase of the control experiment in figure 4. Within approximately 20 seconds after resumption of air breathing the post-hypoxemic pressor effect appears; it is more marked than in the control experiment in figure 4.

FIG. 6. EFFECT OF EPINEPHRINE INJECTED, as in figure 3, during the early depressor phase of the nitrogen breathing period. Conventions as in figures 3 and 4. Epinephrine was injected during the depressor phase of hypoxemia. It caused a slight pressor effect, less marked and more transient than in figures 3 and 5 (compare with fig. 4). The post-hypoxemic pressor effect was more marked than in the control experiment in figure 4 and more protracted than when epinephrine was injected earlier during nitrogen breathing as in figure 5.

FIG. 7. EFFECT OF EPINEPHRINE INJECTION, as in figure 3, during the late depressor phase of the nitrogen breathing period. Conventions as in figures 3 and 4. Epinephrine was injected during the late depressor phase of hypoxemia. It caused no pressor effect during the hypoxemia period (contrast with figures 5 and 6). It may have had a masked depressor effect. A marked post-hypoxemic pressor effect occurred, greater than in the control experiment in figure 4 and also greater than in experiments where epinephrine was injected earlier during hypoxemia.

with fig. 4). In 43 trials in which epinephrine was injected into the femoral vein or left ventricle during the hypoxemic phase, a heightened post-hypoxemic rise averaging 136 mm. Hg and ranging from 39 to 192 mm. Hg above the reference level was seen. This compared with a post-hypoxemic rise of about 30 to 50 mm. Hg in those animals not receiving epinephrine. Six trials showed no increased post-hypoxemic rise after epinephrine. These six trials included four trials in which no recovery occurred and in which the circulatory failure was rapidly progressive, leading to death in a few minutes.

When epinephrine was injected early during the post-hypoxemic pressor period a very high pressure level was obtained without any significant latent period (see table 3), indicating a summation of the post-hypoxemic and epinephrine pressor effects.

TABLE 3. PRESSOR ACTION OF INJECTED EPINEPHRINE IN NORMAL, HYPOXEMIC AND POST-HYPOXEMIC STATES

CONDITION	NO. OF EXPERIMENTS	RESPONSE TO 0.5 MGM. EPINEPHRINE INJECTED INTO FEMORAL VEIN
Control	9	immediate rise, averaging 166 mm. Hg[1]
During early hypoxemic pressor phase	21	immediate rise, averaging 63 mm. Hg[1]
During late hypoxemic depressor phase	19	little or no response until return to air breathing after which pressor effect occurs in 15–75 seconds and averages 140 mm. Hg[1]
Post-hypoxemic phase	5	immediate rise, averaging 204 mm. Hg[1]

[1] Above control level.

In order to eliminate the influence of variations in venous transport to the heart, injections were made directly into the left ventricle at various times during nitrogen breathing in five animals. In these experiments, in contrast with femoral injection, some pressor activity could be demonstrated during the early hypoxemic depressor phase (fig. 6). In general, however, the arterial pressure responses to such intracardiac injections were similar to those observed with intravenous injection. Little or no pressor responses were obtained when epinephrine was injected into the left ventricle in the late hypoxemic depressor phase (fig. 7). Figures 3 to 7 illustrate the response to intraventricular injection of epinephrine in a single animal after varying periods of nitrogen breathing as compared to that after the injection of epinephrine during air breathing and as compared to the changes of hypoxemia alone.

DISCUSSION

We have presented a detailed study of the effects of acute severe hypoxemia on the blood pressure. The pressor effects seen during the hypoxemic and post-hypoxemic period have been observed previously (1–4). Earlier studies have, for the most part, been carried out on animals in which hypoxemia was induced by the breathing of air with low oxygen tensions as in the decompression chamber with mixtures of gases. In none, however, has there been a quantitative analysis of the effects of the more fulminating form of hypoxemia which we have produced.

It is clear that rises in blood pressure occur fairly constantly during early hypoxemia. With our technique this phase lasts for only a minute or so after the institution of nitrogen breathing. Following this, the blood pressure begins to fall and this depressor phase, if permitted to continue, results in death within a few minutes. Reinstitution of air breathing after various durations of the hypoxemic depressor period results almost always in an immediate and marked blood pressure rise. This rise is usually greater than the pressor effect seen during the early phase of nitrogen breathing. Our results indicate that the degree of this post-hypoxemic pressor effect is related to the duration of the hypoxemic depressor phase.

Our results may be interpreted as indicating that the hypoxemia results in sympathetic stimulation and the liberation of a pressor material which manifest themselves at once in the rise in arterial pressure. The rise with early hypoxemia appears to be due mainly and perhaps wholly to sympathetic stimulation (4, 5). However, as the hypoxemia is continued, a point is reached at which the pressure begins to fall. This fall in pressure is apparently due to a failure of the circulatory system to respond to pressor states since subsequent re-oxygenation of the animal results in post-hypoxemic blood pressure rise. The magnitude of this rise in pressure is independent of changes in the heart rate although these may occur (fig. 1).

The fall from the hypoxemic peak is so rapid that the reference level is reached in about 30 seconds. While the rapid fall begins during a time when the heart is not grossly dilated and appears to be pumping vigorously, most authors attribute the fall solely to a diminished cardiac output (4, 6, 7). This point of view has been espoused on the basis of cardiometric and plethysmographic studies and analyses of pulse pressure contour curves, but it has not been concurred in by all (2). It is undoubtedly true that in the late stages of hypoxemia, heart failure is present as is indicated by the obvious marked dilatation and the diminished stroke volume. However, further attention must also be focused upon the failure of the peripheral blood vessels to maintain their tone as a possible participating mechanism. In favor of this viewpoint is a report that the isolated leg perfused with anoxic blood shows a vasodilatation which gives way to vasoconstriction when the leg is re-oxygenated (3). Further suggestive evidence is present in the work of Dale and Richards (8) who reported that histamine caused no vasodilatation in the presence of blood or of gum-Locke solution (low oxygen content) containing epinephrine, although it did cause vasodilatation when epinephrine and blood (higher oxygen content) were present together in the perfusing fluid. Because of the factor of cardiac failure which appears late in severe hypoxemia the rôle of the peripheral vessels cannot be assayed with certainty from our data.

The post-hypoxemic pressor effect persists after section of the cervical spinal cord (4, 10, 11). Stavraky (4) has reported that a substantial post-hypoxemic rise appears in adrenalectomized animals. The relationship between the duration of the hypoxemic depressor phase and the degree of the post-hypoxemic rise which we found would indicate the accumulation of pressor material during the hypoxemic depressor phase which requires the presence of oxygen in the tissues for it to exert its pressor action. In the absence of oxygen this material does not lose its potential pressor effect, but accumulates so that its action is seen only after the re-oxygenation of the tissues.

During the early stages of the hypoxemic pressor phase, epinephrine caused a rise in pressure which was aborted with the development of a greater degree of hypoxemia. When the epinephrine was given intravenously during the hypoxemic depressor phase, no rise in pressure was observed until the reinstitution of air breathing. During increasing hypoxemia a progressive diminution of pressor response was seen. When oxygen was again made available to the tissues, the previously injected epinephrine manifested itself by a rise in pressure which was usually considerably greater than that seen in the control. In this way, the epinephrine acted to summate with the pressor material produced during the hypoxemic depressor phase.

Our results suggest that epinephrine requires the presence of oxygen in the blood stream and tissues in order to exert its pressor action. In the absence of oxygen, it fails to act, but apparently it is not destroyed at a significant rate. This dependence on oxygen for physiological action may be considered as supporting the contention of Blaschko and Schlossman (9), based on *in vitro* work with amine oxidase, that the degradation of epinephrine requires oxygen. However, it is not yet clear whether the fall in pressure during the hypoxemic depressor phase and the inhibition of the pressor action of epinephrine are due to an inability of the substances to act, or upon the failure of the heart and circulation as a result of the severe hypoxemia produced.

Aside from their strictly physiological implications, our results are pertinent to the treatment of certain emergency conditions involving hypoxemia. It is common practice to inject epinephrine into the circulation or even directly into the heart in conditions of severe hypoxemia such as after drowning. Our results indicate that such injected epinephrine may fail to have the desired effect because of the low oxygen tension in the tissues. Further it appears that an epinephrine-like pressor material is elaborated during the period of hypoxemia and that this substance remains in the blood and tissues until adequate oxygen again reaches the tissues. The introduction of further pressor materials in such situations would appear to serve no useful purpose. Oxygenation of the tissues is demanded before such endogenous or exogenous pressor materials can exert their beneficial effects. Furthermore, the intravenous or intracardiac injection of epinephrine in severe hypoxemia may be deleterious since both hypoxemia and epinephrine can predispose the heart to ventricular fibrillation.

SUMMARY

1. Acute severe hypoxemia produced by nitrogen breathing in the anesthetized dog caused a gradual rise in arterial pressure, reaching a maximal level in 60 to 90 seconds. Continuance of nitrogen breathing beyond this time is followed by a rapidly progressive fall in pressure to shock levels.

2. Re-oxygenation after a period of falling blood pressure results in a marked rise in pressure. This post-hypoxemic pressor effect is attributed to the accumulation of pressor materials which do not act in the absence of oxygen.

3. Epinephrine injected during the hypoxemic depressor phase acts similarly in that its pressor action is held in abeyance until the animal is re-oxygenated.

4. Some physiological and clinical aspects of these findings are discussed.

REFERENCES

1. MATHISON, G. C. J. Physiol. **42:** 283, 1911.
2. KAYA, R. AND E. H. STARLING. J. Physiol. **39:** 346, 1909.
3. BEYNE, J. Compt. rend. Soc. de biol. **136:** 399, 1942.
4. STAVRAKY, G. W. Canad. J. Research, Sect. E. **23:** 174, 1945.
5. SURTSHIN, A., A. VAN LOO AND L. N. KATZ. The role of the adrenal gland in the arterial pressure response to severe hypoxemia. In preparation.
6. WIGGERS, C. J. Ann. Int. Med. **14,** 1237, 1941.
7. SANDS, J. AND A. C. DE GRAFF. This Journal **74,** 416, 1925.
8. DALE, H. H. AND A. N. RICHARDS. J. Physiol. **52,** 110, 1918.
9. BLASCHKO, H. AND H. SCHLOSSMAN. J. Physiol. **39,** 346, 1909.
10. LUCHSINGER, B. Arch. f. de. ges. Physiol. **16,** 510, 1878.
11. KONOW, H. G. AND T. STENBECK. Skand. Arch. f. Physiol. **1,** 408, 1889.

BLOOD GLUTATHIONE IN THE BOVINE[1]

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Numerous reports suggest the significance of glutathione in the physiological and pathological processes of the human being and of various animals. Some normal blood levels of this compound in reduced form reported in the literature for various species are: human beings, 34.5 (1); horse, 32.6 (2); ass, 36.4 (2); mule, 36.1 (2); Bactrian camel, 24.2 (3); dromedary, 25.4 (3); hybrids of Bactrian camel and dromedary, 26.4 (3); and rabbits of the breeds Champagne, 47.6 (4), Flemish Giant, 40.0 (4), German Ermine, 45.6 (4), and scrubs, 43.7 mgm. per cent (4). According to one report (1) the normal content of oxidized and total glutathione in human blood is 13.5 and 47.3 mgm. per cent, respectively.

Although Harding and Cary (5) were able to demonstrate the presence of glutathione in the blood of the bovine, the blood levels in healthy cattle apparently have not been studied previously. In view of the suggested significance of glutathione in other species and the lack of data on the bovine, this study was initiated for the purpose of ascertaining the concentration of this substance in both sexes of healthy cattle of various ages and performing different physiological functions. The possible effect of diet upon the levels of glutathione in the blood of these animals was also investigated.

EXPERIMENTAL

Blood glutathione was measured approximately monthly for 15 months in 12 bulls constituting two groups in a feeding experiment and producing semen at varying rates, and in 49 cows and their 50 calves immediately following parturition. The cows composed four groups in an experiment designed to study the effects of various mineral supplements in the prepartal diet. Reduced, oxidized and total glutathione were determined in whole blood according to the method outlined by Woodward and Fry (6).

RESULTS AND DISCUSSION

Diet and blood glutathione. In a study of the relative merits of a simple and a complex concentrate feed mixture for breeding bulls, no differences were found in the reduced, oxidized and total glutathione content of the whole blood of bulls receiving equal quantities of digestible nutrients in these diets. Since the complex diet provided much greater quantities of total protein (from a variety of sources), crude fat, calcium, phosphorus, manganese, iron, cobalt, copper, vitamins A and D and other factors than did the simple mixture, a dietary effect upon the level of blood glutathione in the bull seems remote. Since dietary influences appeared to be lacking, the data for the two groups of bulls were combined and are shown in table 1.

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In an experiment in which calcium and various trace elements were fed to cows for at least two months prepartum, these elements did not elicit perceptible effects upon the blood glutathione of cows or their newborn calves as determined in blood procured immediately following parturition.

Relation of age and colostrum ingestion to blood glutathione. The data presented in tables 1 and 2 demonstrate that the concentration of reduced and total glutathione in the blood of the bovine varies with age, whereas the quantity of oxidized glutathione remains within fairly restricted limits. Since the blood of newborn calves (average age less than one hour) contains significantly greater quantities of both reduced and total glutathione than their dams (table 2) or the other animals studied (table 1), it would appear that these levels decline sometime between birth and 18 months of age. The data further indicate that an increase in the levels of these constituents accompanies aging of bulls from 18 to 33 months (table 1). The cows used in this study ranged from 29 to 155 months of age. When these animals were grouped according to age in 10-month intervals, no mathematically significant differences were found between groups. This observation would indicate that the levels of glutathione remain fairly

TABLE 1. RELATIONSHIP OF AGE OF BULLS TO BLOOD GLUTATHIONE LEVELS
Mean ± std. error

AGE	NO. CASES	GLUTATHIONE (MGM. %)		
		Reduced	Oxidized	Total
mo.				
18–21	24	28.10 ± .73	5.74 ±.42	33.81 ± .78
21–24	35	30.71 ± .90	8.40 ±.34	39.11 ± .85
24–27	47	34.93 ± .72		
27–30	22	34.90 ±1.22		
30–33	33	41.51 ± .93	6.34 ±.45	47.11 ±1.34

TABLE 2. BLOOD GLUTATHIONE OF COWS AND THEIR NEWBORN CALVES
Mean ± std. error

BOVINE	NO. CASES	GLUTATHIONE (MGM. %)		
		Reduced	Oxidized	Total
All cows	49	38.29 ± 1.26	6.79 ± 0.82	45.08 ± 1.28
All newborn calves	50	56.36 ± 2.10	7.08 ± 0.86	63.44 ± 1.93
Newborn bull calves	26	55.53 ± 3.64	6.09 ± 1.20	61.62 ± 3.13
Newborn heifer calves	24	57.19 ± 2.14	8.07 ± 1.23	65.26 ± 2.28
Calves (no colostrum)	43	57.82 ± 2.12	7.21 ± 0.96	65.03 ± 1.78
Calves (colostrum)	7	47.78 ± 6.74	6.33 ± 1.91	54.11 ± 6.38

fixed at maturity or that the picture relative to the influence of age on the blood level of glutathione is obscured by the physiological changes occurring in the cow at the time of parturition.

The figures shown in table 2 for seven calves which had ingested colostrum before blood was obtained for analysis, at an average age of 4.5 hours, would indicate that both the reduced and total glutathione concentrations decrease rapidly following colostrum ingestion. Perhaps the greatest proportion of the decrease in glutathione concentration from birth to 18 months of age occurs during the very early life of the calf. Similar levels of reduced, oxidized and total glutathione were found in the blood of the newborn calf regardless of sex.

Effect of semen production upon the reduction of oxidized glutathione in blood by metallic zinc. It will be noted that no values for oxidized or total glutathione are given in table 1 for bulls 24 to 30 months of age. Although an attempt was made to measure these constituents, some factor(s) appeared to be present in the blood which precluded a successful determination. The difficulty seemed to be associated with a brownish discoloration of the filtrate, a lack of bubbling when zinc dust was added to the acid filtrate (during which process-oxidized glutathione is normally reduced, allowing its quantitative measurement) and, in a few samples, some slight oxidation of reduced glutathione. These symptoms suggested that the failure to reduce oxidized glutathione and, thereby, the failure to measure total glutathione were due to some fault of the zinc or acid used. The use of a number of different lots of zinc and sulfosalicylic acid and different inorganic acids, however, failed to remedy the condition. During this period (24–30 months of age) the bulls were yielding semen at fairly high rates (average per bull, 1.41 ml. per day). When these animals were rested, it was found that the total and oxidized glutathione could again be measured. Therefore, it appeared that some property of the blood during the periods of high semen yield inhibited the reduction of oxidized glutathione by zinc, although the possibility of hydrolysis of oxidized glutathione to cystine (7) cannot be eliminated. A similar condition was reported by Dohan and Woodward (8) and Woodward (9) for human blood. These workers (8) using electrolytic reduction showed that oxidized glutathione is not bound by the proteins of blood as suggested by Quensel and Wachholder (10) as the reason for the failure to recover oxidized glutathione in acid filtrates, following zinc reduction. Woodward (9) and Dohan and Woodward (8) proposed that the blood contributes something to the filtrate which leads to inhibition of zinc reduction. This difficulty appears to be obviated by electrolytic reduction (8).

SUMMARY

A study was made of the levels of reduced, oxidized and total glutathione in the blood of breeding bulls and in that of cows and their newborn calves immediately following parturition.

Although diet within the limits employed in this study did not influence the concentration of glutathione of either form in bulls' blood, the levels of reduced

and total glutathione gradually increased as age progressed from 18 to 33 months. Following parturition, similar levels of glutathione were found in the blood of cows ranging from 29 to 155 months of age.

Calcium fed two months prepartum as the only supplement and in conjunction with various trace elements in a basic dry-cow feed did not significantly affect the levels of glutathione of either state in the blood of the dams or their newborn calves as determined in blood taken soon after parturition. The blood of the calves, however, contained significantly greater quantities of reduced and total glutathione than that of their dams or of any of the animals studied. Calves having ingested colostrum before blood was obtained for analysis, at an average age of 4.5 hours, showed markedly lower concentrations of reduced and total glutathione than those which had not received this food. These data suggest a rapid decline in blood glutathione during the early life of the calf. Similar levels of glutathione were found in the blood of newborn calves regardless of sex.

Age, sex and diet did not appear to be related to the level of oxidized glutathione, as the concentration of this compound was similar for all animals studied. The failure to measure oxidized glutathione in the blood of bulls yielding large quantities of semen was attributed to changes occurring in the blood under these conditions which inhibit the reduction of oxidized glutathione by metallic zinc.

REFERENCES

1. Lovtskii, E. A. and L. I. Dikhovichnaya. J. Med. Ukraine **11:** 267, 1941; Chem. Abs. **37:** 175, 1943.
2. Patrusev, V. I. Izv. Akad. Nauk SSSR. (Otd. mat.-est., Ser. biol) 929, 1938. An. Breed. Abs. **7:** 100, 1939.
3. Patrusev, V. I. Izv. Akad. Nauk SSSR. (Otd. mat.-est., Ser. biol.) 917, 1938. An. Breed. Abs. **7:** 146, 1939.
4. Filippova, L. A. Usp. zooteh. Nauk **5:** 144, 1937. An. Breed. Abs. **7:** 32, 1939.
5. Harding, T. S. and C. A. Cary. Proc. Soc. Exp. Biol. Med. **23:** 319, 1926.
6. Woodward, G. E. and E. G. Fry. J. Biol. Chem. **97:** 465, 1932.
7. Woodward, G. E. Biochem. J. **33:** 1171, 1939.
8. Dohan, J. S. and G. E. Woodward. J. Biol. Chem. **129:** 393, 1939.
9. Woodward, G. E. Personal communication, 1947.
10. Quensel, W. and K. Wachholder. Z. physiol. Chem. **231:** 65, 1934.

EFFECT OF ADMINISTERING AGENIZED AMINO ACIDS AND WHEAT GLUTEN TO DOGS[1]

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During the past twenty-five years different theories have been suggested as to the cause of canine hysteria or so-called 'running fits.' A comprehensive survey of the problem, made by Hewetson in 1936 (1), revealed that no definite cause of the disease was known at that time. Arnold and Elvehjem (2) suggested that a deficiency might exist in the ration of dogs which developed fits. In 1944, however, Wagner and Elvehjem (3) demonstrated that running fits were due to a toxicity brought on when high levels of wheat gluten were fed to dogs. A report by Mellanby (4) showed that fits occurred when wheat flour was treated with a commercial bleaching agent, nitrogen trichloride (agene). Untreated flour fed as long as sixteen weeks caused no untoward reactions. Moran (5), Newell *et al.* (6) and Silver *et al.* (7), confirmed Mellanby's work and also showed that other food products such as casein, zein, wheat gluten, rye and gelatin can induce fits when they are treated with nitrogen trichloride.

Although the general symptoms of running fits have been recognized for a long time, no intensive investigations have been undertaken to determine if physiological changes take place when the dog develops the seizures. Arnold and Elvehjem (2) were unsuccessful in preventing the occurrence of fits when lysine dihydrochloride was administered intravenously. Supplements of thiamine hydrochloride given orally or intraperitoneally and oral supplements of choline chloride were found by Morgan and Groody (8) and Wagner and Elvehjem (3) to be ineffective in preventing fits. The latter group (3) and Mellanby (4) reported that a vitamin A deficiency did not cause fits and that supplements of the vitamin had no effect in the prevention or cure of the disease.

The experiments reported here were begun in an attempt to determine if any changes occur in the blood of dogs when they develop fits. Results obtained when agene-treated products were fed to dogs, previously 'sensitized' with agenized flour, are also included.

EXPERIMENTAL

Weanling mongrel dogs up to six months of age were used in these experiments. Each animal was dewormed, dusted with DDT, immunized against distemper and then transferred to a basal control ration (6) before use in the subsequent experiments. The animals were kept in a heated, lighted room in individual heavy wire mesh cages, the floors of which were concrete and covered with wood shavings. Under these conditions it was possible to make close observations of each animal and to follow its daily food consumption.

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The general procedure followed, during the experiments on blood physiology, was to maintain healthy dogs on the basal control ration for a week or more during which time 10 cc. blood samples were removed daily before feeding. Agenized wheat gluten was then substituted in the ration in place of casein. Blood samples were taken for several days after the onset of fits was first observed. As is shown in the tables it was often possible to obtain samples when a dog was in the convulsive state. At the end of each experiment, dogs which had received fit-producing rations were transferred to the basal control ration for a recovery period of at least a week. By this procedure it was possible to use each dog for several experiments.

All blood was drawn from the radial vein. The prothrombin time of the plasma was determined by the method of Campbell *et al.* (9) and was estimated by the number of seconds required for a clot to form in 12.5 per cent plasma containing known amounts of calcium and thromboplastin. The thromboplastin was prepared at regular intervals from normal rabbit brain. Blood samples for the determination of serum calcium obtained in a dry, unoxalated syringe were carried out according to the method of Clark and Collip (10). To prevent clotting of samples, oxalated or heparinized blood was used in all other analyses. Hemoglobin determinations were carried out by the method of Evelyn (11). Routine red and white cell counts were made and hematocrit values were obtained with Wintrobe tubes. Blood sugar values were determined by the method of Folin (12).

Supplements of calcium pantothenate, potassium, magnesium, selenium and treated and untreated amino acids were administered orally, intravenously or subcutaneously as described later in an effort to suppress or induce fits. Mixtures of the more insoluble amino acids were put in solution with 2N NaOH and adjusted to pH 7.3 with HCl. To 'sensitize' the dogs, rations were fed which contained agenized wheat gluten or flour. When fits developed the basal ration was substituted and the dogs were allowed to return to their normal state. Tests were then carried out to see if the sensitized animals developed fits when non-agenized protein products or amino acids were fed. At the same time, control dogs which had never received agenized materials were tested in a similar manner. All amino acid supplements were fed daily at a rate equivalent to the amount of that particular amino acid which would be present in 40 grams of wheat gluten. A dog normally consumes 200 grams of the gluten ration (which contains 40 grams of wheat gluten) per day.

In the water retention studies, dogs were kept in all-metal metabolism cages before the gluten ration was begun. Weights were recorded, measured amounts of water consumed, urine excretion, and food consumption tabulated daily.

RESULTS

In tables 1 and 2 data are presented which summarize the experiments made on the composition of the blood of the dogs. These animals were selected from a larger group of dogs tested and for the sake of brevity the results are presented as a representative sampling of the data obtained. No appreciable changes were found in the values for hemoglobin, hematocrit, red blood cell count, white blood cell count, calcium, glucose, prothrombin, phosphorus, sulfur or vitamin C. Vitamin C values tended to vary but no definite trend was indi-

TABLE 1. EFFECT OF AGENIZED WHEAT GLUTEN ON THE BLOOD COMPOSITION OF DOGS

DOG	DATE	WT.	HEMOGLOBIN	HEMATOCRIT	RED BLOOD CELL	WHITE BLOOD CELL	Ca	GLUCOSE	PROTHROMBIN TIME	COMMENT
		kgm.	*mgm. per cent*	*mgm. per cent*	*Mil/cmm.*	*No/cmm.*	*mgm. per cent*	*mgm. per cent*	*sec.*	
128										
Basal ration	3/31	5.30	11.07	32.0	2.17	8600	11.6	97	26	
	4/2	5.20	9.34	36.0	3.36	6120	10.6	108	26	
	4/4	5.30	9.34	34.5	3.25	6560	9.4	107	26	
Gluten ration	4/7	5.20	9.80	34.5	2.04	7200	12.3	105	22	Fits observed
	4/8	5.20	9.88	33.0	1.85	6240	11.9	105	23	
	4/9[1]	5.20	9.88	34.0	2.43	5680	11.7		25	
132										
Basal ration	2/11	4.05	9.26	26.4	3.42	6425	11.6	97	26	
	2/12	4.15	7.80	27.3	1.98	4560	10.5	103	28	
	2/13	4.05	9.26	27.8	3.85	4120	10.6	108	26	
Gluten ration	2/16	3.80	8.05	21.8	2.93	4700	11.9	104	24	Fits observed
	2/17[1]	3.75	10.87	29.4	3.65	6000	12.3	105	26	
	2/19	3.85	9.06	23.7	2.88	6400	11.9	105	25	
137										
Basal ration	3/20	5.10	9.88	27.0	2.12	8720	10.7	102	30	
	3/21	5.10	8.74	27.0	2.67	8000	10.5	104	27	
	3/22	5.15		28.5	2.02	6280	11.8	104	29	
Gluten ration	3/26	4.95	9.34	31.5	3.53	5680	11.7	90	37	Fits observed
	3/27	4.95	9.34	33.0	1.92	7360	10.1	81		
	3/29[1]	4.90	7.48	26.5	2.38	5120	11.5	85	34	
136										
Basal ration	2/16	2.05	8.68	22.7	3.21	10360				
	2/17	1.90	7.04	17.0	2.49	7920				
Gluten ration	2/19	2.10	7.90	17.5	2.38	7120				Fits observed
	2/21[1]	1.90	8.88	21.6	3.59	9870				

[1] Bled during seizure.

cated. Even when blood was withdrawn during a seizure, there was no change beyond the normal limits.

TABLE 2. EFFECT OF AGENIZED WHEAT GLUTEN ON THE BLOOD COMPOSITION OF DOGS

DOG NO.	DATE	MAGNESIUM	POTASSIUM	PHOSPHORUS	SULFUR	VITAMIN C
Basal ration						
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
21	8/14	1.8	15	38	1.4	0.75
242	8/14	1.3	14	34	—	1.10
243	8/14	1.9	14	36	1.5	1.10
Gluten ration						
21	8/23	2.9	40	41	1.6	0.40
242	8/23	3.3	26	32	1.3	1.40
243	8/23	2.6	18	39	1.4	0.75

TABLE 3. WATER RETENTION STUDIES OF DOGS MAINTAINED ON THE BASAL RATION (20 PER CENT CASEIN), AND ON A RATION CONTAINING 20 PER CENT WHEAT GLUTEN

DOG NO.	DATE	WEIGHT	WATER CONSUMED	WATER RETENTION
		kgm.	*cc.*	*cc.*
128				
Basal ration	3/10	4.00	480	120
	11	3.85	600	400
	12	3.95	370	110
	13	4.20	620	320
Gluten ration	18	4.65	950	250
	19	4.60	940	250
	20	4.75	900	400
	21	4.75	1000	420
137				
Basal ration	3/10	4.45	670	470
	11	4.65	900	400
	12	4.70	490	200
	13	4.70	690	280
Gluten ration	18	4.95	1000	400
	19	5.10	860	390
	20	5.10	710	250
	21	5.15	860	390

Results obtained for magnesium and potassium when dogs were fed agenized wheat gluten showed an increase of 22–166 per cent above the normal values. It will be observed that for each dog tested, there was a marked increase in these values when agenized gluten was fed.

In table 3 are summarized the results obtained with representative animals in the water retention studies. No variation in the water balance was noted when agenized gluten was fed in comparison to the water balance observed when the animals were fed the basal ration. Although the absolute water consumption and water retention had increased somewhat by the end of gluten feeding, it must be remembered that young dogs were used and a gradual weight increase was evident in all animals.

Since large amounts of wheat are grown in areas where selenium is present in the soil, it was earlier considered that running fits might be due to a toxicity of this element. Anderson and Moxon (13) have reported that Se toxicity in dogs is not observed until it is injected at a level of 1.5–2.0 mgm. per kilo of body weight. Analyses of several samples of wheat gluten gave values for Se far below those causing toxic effects in dogs when a comparable amount of gluten was included in a ration. As calculated from the values of Se in the gluten ash, 0.2 mgm. of Se would be consumed by a dog in a five-day period while developing fits on a gluten ration. One dog was thus injected subcutaneously with 0.2 mgm. Se as Na_2SeO_3. No toxic symptoms were observed during the next three days at which time one mgm. of Se per kilo was administered subcutaneously. The dog was normal the next two days. On the sixth day 2.0 mgm. of Se per kilo were given subcutaneously. Forty-five minutes later the dog was ataxic and its breath was heavy with a garlic odor characteristic of Se poisoning. The dog became progressively weaker and was found dead the next day. At no time were there any symptoms of running fits.

To determine if magnesium has any effect in preventing fits two dogs having seizures were given 120 mgm. Mg, as $MgCl_2$, intravenously. Twelve hours later the animals were still showing fits and were then given one gram, each, of Mg subcutaneously. Convulsive seizures continued for three more days, followed by the death of one animal.

One dog which had several attacks of fits was given 100 mgm. of potassium, as KCl, intravenously. No decrease in seizures was observed and the procedure was repeated the following day. Partial blindness, characteristic of an advanced stage of fits, was still evident two days later. Ataxic hind legs also indicated that K has no therapeutic effect in running fits.

A dog with fits was given 15 mgm. of calcium pantothenate intravenously followed by a second injection of 20 mgm. the next day. Seizures and all typical symptoms of fits were observed the second and third day after treatment. No improvement with calcium pantothenate was indicated.

In view of the results obtained by Silver *et al.* (7), using agene-treated amino acids to induce running fits, it was decided to study the effect of both orally and intravenously administered amino acids. Two dogs, while on the basal ration, were given seven grams of non-agenized glutamic acid daily. The amino acid, dissolved in dilute HCl, was fed by stomach tube for three days. No supplements were administered the next two days. For the next three days, glutamic acid, treated mole per mole with agene, was fed by stomach tube at the rate of seven grams per day. Both animals remained normal throughout this experimental period.

The same procedure was used to test the effect of untreated and treated tyrosine when 1.25 grams of the amino acid were fed by stomach tube to two dogs. No toxic effects were observed in either animal.

One hundred grams of dl-methionine, treated with agene at 50 grams per kilo of methionine, were incorporated in two kilos of basal ration, at the expense of sucrose. One dog consumed 100 grams of this ration a day for twenty days without showing any symptoms of running fits. Methionine sulfoxide was then prepared according to the methods of Toennies and Kolb (14 and 15) and fed in the basal ration at 0.6 per cent to one dog for 10 days. No reaction was observed. On the eleventh day, 2.4 grams of methionine sulfoxide were injected intravenously into this animal. An electroencephalogram record was taken at frequent intervals for the next hour but no changes in the E.E.G., characteristic of fits, were recorded.

Two young 'non-sensitized' dogs were fed 100 grams of the basal ration per day, containing one per cent highly agenized cystine, for a period of eight days. There was no evidence of running fits. A dog, previously 'sensitized' when fed the agenized gluten, received by intravenous infusion a mixture of the following highly agenized amino acids: three grams cystine, two grams methionine, two grams tryptophane. There were no fits during or after the test.

Mixtures of each of the following highly agenized amino acids have been incorporated into the basal ration and fed for a period of ten days: 16 grams cystine and 16 grams valine, 16 grams cystine and 32 grams valine, 16 grams cystine and 7 grams serine, 16 grams cystine and 14 grams serine. Each mixture was fed to a 'non-sensitized' and to a 'sensitized' dog but in no instance were any fits indicated or observed.

Two dogs, one 'sensitized' and one 'non-sensitized' were each given daily intravenous injections of two grams of agenized cysteine for a three-day period. No reaction was observed in either dog. Cysteic acid, an oxidation product of cysteine, was given I.V. at the rate of 1.25 grams per day for two days to two dogs. Neither the 'non-sensitized' nor the 'sensitized' animals came down with fits.

One gram of agene-treated tryptophane was fed by stomach tube to a young dog. A second animal received two grams of the material by stomach tube. Both dogs remained normal.

A mixture of amino acids, containing the amino acids known to exist in wheat gluten, were heavily treated with agene and then incorporated in the basal ration at the expense of half the casein. This ration was fed to two dogs for 12 days without any evidence of fits. The animo acids consumed daily by each dog were equivalent to those present in 40 grams of wheat gluten. Repeated experiments have shown that if a dog consumes 80 grams of gluten, treated at 20 grams per cwt. with agene, the animal will consistently develop severe fits.

Five grams of glutathione were treated with 2 grams of agene and then incorporated into the basal ration at a level of 0.5 per cent. One dog fed this ration for eight days remained normal throughout the test.

DISCUSSION

Although there was some variation between dogs in the case of each blood component determined, the data clearly show that those changes observed were consistent for all animals. There was an increase in the blood magnesium of dogs which developed fits, but intravenous injection of the element into animals fed a fits-producing ration did not precipitate seizures. Orent, Kruse and McCollum (16) have reported that dogs with a magnesium deficiency exhibit hyperexcitability and convulsions. Potassium also increased in the blood when agenized gluten was fed but it would seem that although there is a notable increment of this element when a dog develops seizures, the high blood potassium is not important in running fits. Even intravenous injections of KCl to dogs exhibiting intermittent fits did not increase the frequency or severity of the seizures. Earlier, Melnick and Cowgill (17) showed that the blood non-protein nitrogen values of dogs were essentially normal before and after the administration of gliadin. They concluded that no significant uremia existed when dogs develop running fits. Results of the present study on the blood and on the water retention of dogs tend to support that conclusion.

Schaefer, McKibbin and Elvehjem (18) have described a sudden prostration, convulsions, and gastrointestinal symptoms in pantothenic acid deficient dogs. Calcium pantothenate in this case did not have a therapeutic effect when it was administered to dogs having intermittent seizures.

Silver *et al.* (7) have reported that running fits can be induced in dogs when the animals are first 'sensitized' by feeding agenized flour rations and then injected with agenized amino acids. It must be emphasized that the seizures only occurred, on injection of amino acids, after the dogs had first been on a fits-producing ration for periods up to two weeks. Dogs in such a condition might easily develop a convulsive seizure when any foreign material is injected into the blood stream. In 12 dogs fed a non-agenized flour ration and then injected with agene-treated amino acids, only 4 animals showed E.E.G. changes suggestive of a sub-clinical seizure condition. In no case were fits reported.

The amino acids, cystine and cysteine, reported by Silver *et al.* (7) to be active, have been fed by mixing in ration, by stomach tube and by I.V. injection without causing any indication of even a mild fit. Until clinical convulsions can be precipitated by the toxic product isolated from agenized flour, when fed orally, the identity of the 'running fits' factor must remain in doubt.

SUMMARY

No consistent or significant differences were observed between values for hemoglobin, hematocrit, red blood cell count, white blood cell count, prothrombin time, glucose, calcium, phosphorus, sulfur or vitamin C of the blood of dogs when fed agenized wheat gluten and when fed the basal ration. A definite increase in the blood magnesium and potassium was observed when the agenized wheat gluten was fed.

Selenium toxicity in dogs is not related to the condition of running fits. In-

jection of magnesium, potassium or calcium pantothenate had no effect in preventing or lessening the intensity of the seizures.

Various agenized amino acids were fed as part of a ration, by stomach tube or intravenously, but none of the 28 animals tested developed fits as a result of this treatment.

REFERENCES

1. HEWETSON, H. R. Veterinary Record **48:** 1202, 1936.
2. ARNOLD, A. AND C. A. ELVEHJEM. J. Am. Vet. Med. Assoc. **95:** 303, 1939.
3. WAGNER, J. R. AND C. A. ELVEHJEM. J. Nutrition **28:** 431, 1944.
4. MELLANBY, E. Brit. Med. J. **2:** 885, 1946.
5. MORAN, T. Lancet **2:** 289, 1947.
6. NEWELL, G. W., T. C. ERICKSON, W. E. GILSON, S. N. GERSHOFF AND C. A. ELVEHJEM. J. Am. Med. Assoc. **135:** 760, 1947.
7. SILVER, M. L., R. E. JOHNSON, R. M. KARK, J. R. KLEIN, E. P. MONAHAN AND S. S. ZEVIN. J. Am. Med. Assoc. **135:** 757, 1947.
8. MORGAN, A. F. AND M. GROODY. J. Am. Vet. Med. Assoc. **105:** 406, 1944.
9. CAMPBELL, H. A., W. K. SMITH, W. L. ROBERTS AND K. P. LINK. J. Biol. Chem. **138:** 1, 1941.
10. CLARK, C. K. AND A. L. COLLIP. J. Biol. Chem. **63:** 461, 1925.
11. EVELYN, K. A. J. Biol. Chem. **115:** 63, 1936.
12. FOLIN, A. J. J. Biol. Chem. **67:** 357, 1926.
13. ANDERSON, H. D. AND A. L. MOXON. J. Pharm. and Expt. Ther. **76:** 343, 1942.
14. TOENNIES, G. AND J. J. KOLB. J. Biol. Chem. **128:** 399, 1939.
15. TOENNIES, G. AND J. J. KOLB. J. Biol. Chem. **140:** 131, 1941.
16. ORENT, E. R., H. D. KRUSE AND E. V. MCCOLLUM. This Journal **101:** 454, 1932.
17. MELNICK, D. AND G. R. COWGILL. J. Nutrition **14:** 401, 1937.
18. SCHAEFER, A. E., J. B. MCKIBBIN AND C. A. ELVEHJEM. J. Biol. Chem. **143:** 321, 1942.

THE pH OF GASTRIC MUCOUS SECRETION[1, 2]

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Data at present available on the pH of gastric mucus are so scant that they contribute little to the sum of knowledge about the physicochemical characteristics of this exocrine secretion. Most of the reports (on material from dog, cat and human) state that it is alkaline to litmus, but a few investigators found an acid reaction to this indicator or even to Congo red. In patients with no free acid, Bucher (1) usually obtained pH's (colorimetric) in the range of 6.0 to 6.6, with an occasional value as high as 7.2. Mecholyl, injected subcutaneously into two fasting patients, yielded secretion with a pH (electrometric) of 8.9 (10). When administered iontophoretically, however, this pharmacological agent yielded pH's no higher than 8.3, but only a few values were reported (9). In dogs, the pH of mucus secreted spontaneously from both pyloric and fundic pouches was reported in the range 7.0 to 7.5 by Ivy and Oyama (7); Lim and Dott (8) found the same range for pyloric pouch juice, whereas Takata (11) reported 7.4 to 8.0. Similar material from cats had a pH of 8.4 (colorimetric), as reported by Gamble and McIver (2).

In the present investigation we determined the pH of a large number of specimens of mucous secretion, obtained from pouches of the gastric corpus in dogs. More than 575 values were obtained from both 'spontaneous' and chemically stimulated secretion, thus permitting of a statistical evaluation of the variability manifest in the work of previous investigators. Analysis of the opacity, consistency, and columnar cell content of a majority of these same specimens has already been presented elsewhere (5, 6).

METHODS

Almost all of the specimens of mucus employed for this study were collected from 10 Heidenhain pouch dogs; 15 of them were obtained from three other dogs with gastric explants prepared from the central portion of the greater curvature. Hence, the data relate to secretion from the gastric corpus—not the pyloric region. No experiment was ever started unless the mucosa was free of obvious parietal secretion, as evidenced by a pH of 6.0 or greater. The technique for establishing this condition, as well as all other details of collection procedure, have been described in a previous paper (5). Experiments were performed with

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[1] Preliminary reports of this work were presented before the Federation of American Societies for Experimental Biology in 1941 and 1943. [Hollander and Felberg (3); Hollander and Stein (4)].

[2] This investigation was supported in part by grants from The Altman Foundation of New York and Wyeth Inc.

the following stimuli: 1) gentle massage of the mucosa with a soft rubber catheter for 3 to 5 minutes; 2) distilled water saturated with ether (no greater than six per cent concentration at 30°C.); 3) five per cent aqueous emulsion of clove oil; 4) aqueous eugenol emulsions in concentrations as high as five per cent; 5) ethyl alcohol, 50 per cent; 6) isotonic NaCl solution, 0.17 N; 7) hypertonic NaCl solution, 0.5 N; and 8) distilled water. A set of data on 9) 'spontaneous' secretion was also included. These last specimens were obtained either during experiments without direct stimulation or during preliminary periods preceding the application of one of the aforementioned stimuli. It may be assumed that none of the stimulating fluids exerted any chemical influence on the pH values for the following reasons: a) the stimulating fluid was aspirated from the pouch almost completely before the first specimen of secretion was collected; b) this specimen was discarded whenever it contained any visible amount of the stimulus; c) the stimulating fluids were unbuffered in all cases.

Electrometric pH determinations were made with a glass electrode. In general, no more than three minutes were required to attain constant readings, and these were found to persist for at least 15 minutes when a small series of specimens was studied for this purpose. Multiple determinations on any one specimen agreed within a range of ±0.02 pH, provided the specimens were shaken vigorously to effect homogenization before measurement. Without such shaking, duplicate determinations were liable to differ by as much as several tenths of a pH unit. After the reproducibility of this technique had been established, only single determinations were performed. Since we were concerned with the pH values of mucous secretion, which was presumably free of parietal secretion, all values above 4.00 were included in the tabulation for statistical analysis.

RESULTS

Combined data. A preliminary study of the frequency distribution for all 579 pH values, irrespective of stimulus, is presented in figure 1. The frequency graph is markedly skew, with a long tail on the acid side and a precipitous drop from 8.5 to the upper limit of the range, 9.2. The mean and standard deviations for the entire distribution are 7.65 and 1.08, respectively, but in view of the marked skewness of the graph, and the results of a breakdown of the data by stimuli, these two statistics possess no physiological significance. For purposes of analysis, the distribution was divided into two subranges at 6.8, where the flat tail of the graph terminates. It is noteworthy that more than 85 per cent of the values are in the upper subrange (table 1, line for combined data).

Data for individual stimuli. Next, the data for the total population were broken down according to stimulus, and the following statistics were calculated for each of these groups of data: the mean, its standard deviation ($S.D._M$) as well as that of the distribution (S.D.), and frequencies in the two pH subranges. These statistics are also presented in table 1. The stimuli are arranged in order of increasing mean pH—except for one per cent and two per cent eugenol, for which the difference in concentration has greater statistical significance than the difference in mean pH. Merely by inspection of the data it is apparent that the differences among these means are statistically significant in many instances,

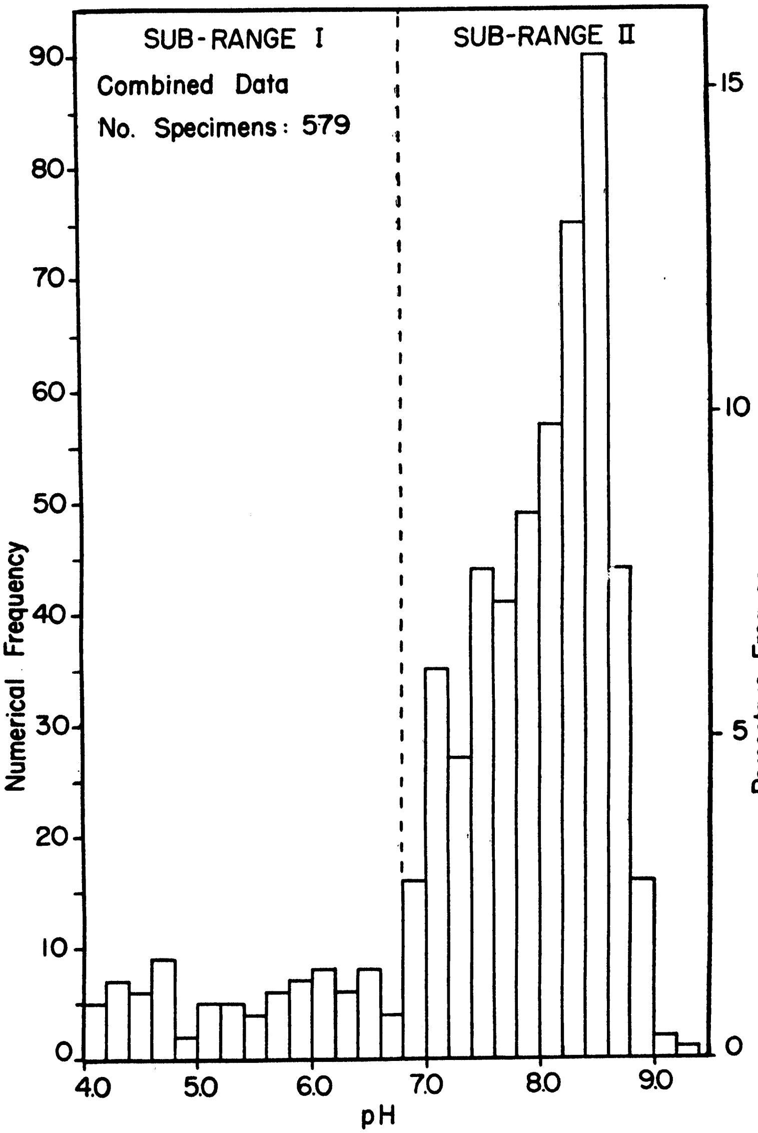
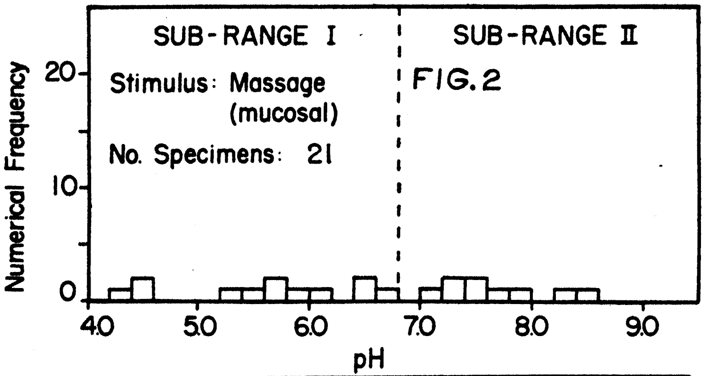


FIG. 1. FREQUENCY DISTRIBUTION for pH values of gastric mucous secretion from Heidenhain pouches (combined data—all stimuli).

but not in all. In nine out of 12 cases, the difference between two *successive* means is only 0.2 pH unit or less; in two others it is about 0.3 and in the third 0.4. Because of this small variation in mean pH from stimulus to stimulus,

TABLE 1. STATISTICAL DATA FOR pH VALUES OF GASTRIC MUCOUS SECRETION

STIMULUS	MEAN pH	S. D.	$S.D._M$	pH RANGE	NUMBER OF SPECIMENS		
					Sub-range 1 (pH 4.00–6.79)	Sub-range 2 (pH 6.80–9.22)	Total
Stimulus—group 1							
Massage (mucosal)	6.48	1.24	0.28	4.20–8.58	12 (57.1%)	9 (42.9%)	21
NaCl (0.17 N)	6.66	1.25	0.38	4.08–8.43	4 (33.3%)	8 (66.6%)	12
H_2O (distilled)	6.83	0.96	0.30	5.19–8.36	5 (45.4%)	6 (54.6%)	11
None (spontaneous)	6.90	1.33	0.14	4.00–9.07	30 (31.6%)	65 (68.4%)	95
Alcohol (50%)	7.02	1.01	0.17	4.20–8.22	8 (21.1%)	30 (78.9%)	38
Eugenol (0.25%)	7.35	1.32	0.31	4.35–8.60	5 (26.3%)	14 (73.7%)	19
Stimulus—group 2							
NaCl (0.5 N)	7.40	0.44	0.13	6.38–7.95	1 (7.7%)	12 (92.3%)	13
Ether (saturated)	7.63	0.88	0.09	4.50–8.83	10 (9.3%)	97 (90.7%)	107
Eugenol (0.5%)	7.97	0.56	0.12	5.70–8.53	1 (4.3%)	22 (95.7%)	23
Eugenol (1%)	8.04	0.58	0.09	6.24–8.65	4 (8.7%)	42 (91.3%)	46
Eugenol (2%)	8.03	0.57	0.07	4.76–8.87	1 (1.7%)	58 (98.3%)	59
Clove oil (5%)	8.10	0.27	0.05	7.28–8.47	0 (0%)	27 (100%)	27
Eugenol (5%)	8.51	0.44	0.04	4.69–9.22	1 (0.9%)	107 (99.1%)	108
Combined data (all stimuli)	7.65	1.08	0.04	4.00–9.22	82 (14.2%)	497 (85.8%)	579



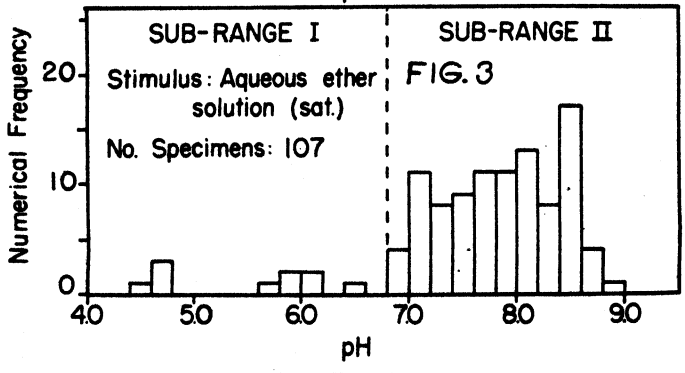


FIG. 2. FREQUENCY DISTRIBUTION for pH values of gastric mucous secretion from Heidenhain pouches (after stimulation of mucosa by massage).

FIG. 3. FREQUENCY DISTRIBUTION for pH values of gastric mucous secretion from Heidenhain pouches (after stimulation of mucosa with saturated aqueous ether solution).

these averages afford no basis for a classification of the stimuli. Such a classification, however, is suggested by the distribution of the data over the pH range. The values for mucosal massage, NaCl (0.17N), distilled water, 'spontaneous,' alcohol (50 per cent), and eugenol (0.25 per cent) extend more or less uniformly over the entire range. These are designated as stimulus-group 1. By way of illustration, the frequency graph for stimulation by mucosal massage is shown in figure 2. The data for the members of stimulus-group 2 show a well-defined concentration above pH 6.8, which is illustrated by the frequency graphs for ether, and eugenol (5 per cent) (figs. 3 and 4). For five of the stimuli in this group the number of specimens with pH's below 6.8 is no greater than one, and n the remainder the number does not exceed 10 per cent. In contrast with this, the proportion of pH values below this boundary value of 6.8 among the stimuli of group 1 varies between 21 and 57 per cent.

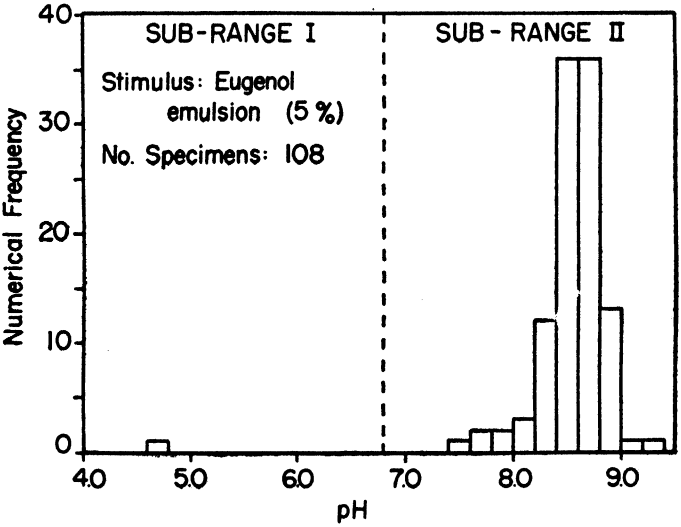


FIG. 4. FREQUENCY DISTRIBUTION for pH values of gastric mucous secretion from Heidenhain pouches (after stimulation of mucosa with 5 per cent eugenol emulsion).

DISCUSSION

The marked skewness of the frequency distribution for the combined data (fig. 1), particularly the long flat tail from pH 4.0 to 6.8, suggests the presence of at least two different types of physiological fluid in what is generally called 'mucous secretion.' The first, with a very low pH value, is probably parietal secretion; the second, with a very high pH value, is true mucus and possibly a mucoid secretion. The peaks at pH 7.1 and 7.4 might result from a third type of fluid, of intermediate pH, e.g., transudate or exudate, but they are hardly high enough relative to the peak at pH 8.5 to constitute evidence of a tri- or bimodal distribution. However, sufficient support for the presence of transudate and exudate is provided by our previous studies to warrant their inclusion as a third possible factor in the interpretation of the present data.

Hence, if we hypothesize that gastric mucous secretion is a mixture of these three type of fluid in varying proportions, then the lower subrange of pH can be considered as containing values characterized by the dominance of parietal secretion, and the upper subrange by the dominance of mucus and mucoid secretions. Inasmuch as the frequency curve for the combined data has only one well-defined mode (pH 8.5), and this occurs in the upper subrange containing 86 per cent of all the values, it may be inferred that mucus and mucoid secretion are by far the most common of all these components. The occurrence of transudate and exudate is more difficult to define; these probably exert a secondary effect in both subranges, but particularly in the lower portion of subrange 2, below 7.8. The role of desquamated columnar cells has been neglected in this hypothesis. It seems likely that in the nondisrupted condition these cells exert little if any hydrogen ion effect. When disintegrated, however, they contribute not only mucus but also cytoplasm, which may be assumed to resemble transudate in its influence on pH.

Turning now to the breakdown of the data by stimulus, it is apparent that every stimulus herein reported yields some 'alkaline' mucus, but the potencies of these agents differ considerably. Actual data for the stimuli in group 2 (from table 1) reveal that 91 to 100 per cent of the specimens induced by eugenol (0.5–5 per cent), clove oil (5 per cent), ether (saturated) and NaCl (0.5N), consist predominantly of true mucus, with a pH of 6.8 or greater. Small amounts of parietal secretion, transudate or exudate may also be present in these specimens. On the other hand the agents of group 1—massage, isotonic saline, distilled water, 'spontaneous' and eugenol (0.25 per cent)—are the poorest mucus stimuli inasmuch as only 43 to 79 per cent of their specimens fall in the upper subrange.

The present study relates to the pH of mucous secretion as it occurs inside a gastric pouch. Since by far the major part of the unoperated viscus contains corpus mucosa, it was deemed essential that the pouches be prepared entirely from this region, rather than from the cardiac or pyloric portions of the stomach. Ultimately, however, we are concerned with the whole stomach rather than any such limited portion of it, but evidence available from the literature suggests that pyloric mucus has essentially the same pH as that from the corpus.

Whether the observations herein reported cast any light on the pH of mucus as it is formed within the cell cannot be stated as yet, because no attempt was made to control or compensate for the possible loss of CO_2 from the specimen, either while it was being collected from the pouch or thereafter. An observation of Wright and Florey (12) is noteworthy in this connection. They reported that the pH of the viscous secretion of the cat's colon, resulting from faradic stimulation of the peripheral ends of the nervi erigentes, is at first 8.3 to 8.4 but increases to 9.1 to 9.2 on exposure to air. In the present work, it was noted that the pH of different portions of any one specimen become essentially identical if the specimen is well shaken. The uniformity so effected was ascribed to 'homogenization' of the specimen, but it may well be that the initial lack of uniformity was a result of irregular loss of CO_2 prior to shaking. Prevention of CO_2 loss before the specimen enters the collection tube is impossible with the usual procedures for collecting the gastro-intestinal secretions. However, correction for such loss may be possible by equilibration of the specimen immediately before the pH is measured, using air containing CO_2 at its partial pressure in alveolar air. Such studies with gastric mucous secretion are now under way.

SUMMARY

Electrometric pHs were determined for 579 specimens of gastric mucous secretion collected from Heidenhain (corpus) pouch dogs. For this purpose, 12 topical stimuli were employed. The range of these pH values was 4.00 to 9.22, with a mean of 7.65 and a standard deviation of 1.08. Division of the data into two subranges of pH revealed that 82 (14 per cent) of the values fell in the lower interval (pH 4.00 to 6.79) and 497 (86 per cent) in the higher interval (6.80 to 9.22). It is inferred that the pH of this latter group is determined predominantly by true mucus, whereas the pH of the former group is influenced in considerable degree by the acid parietal secretion. Division of the data into 13 groups according to stimulus (including spontaneous secretion) revealed a gradual but extensive variation in the mean pHs for the individual groups. However, only aqueous emulsions of clover oil (5 per cent) and eugenol (0.5 to 5 per cent) yielded secretion with mean pHs greater than 8.0. Furthermore, 97 per cent of all the specimens obtained with these stimuli fall in the upper subrange, with pHs greater than 6.8. These observations support the conclusion previously reached by us that clove oil and eugenol are considerably more effective stimuli for the collection of gastric mucus than any others in the series.

The findings herein reported cast no light upon the pH of pure mucus as it is ejected by the surface epithelial cells, because of the loss of CO_2 from the specimen between the time of secretion and the time of pH measurement. Nevertheless, the data herein reported do indicate the pH which may be encountered in gastric pouches, and therefore presumably in the lumen of the intact resting stomach, under the influence of various mucus stimuli. This pH is usually above 7.4 and may even be greater than 9.0—probably the highest value encountered in the living mammalian organism.

REFERENCES

1. Bucher, R. Deut. Z. Chir. **236**: 515, 1932.
2. Gamble, J. L. and M. A. McIver. J. Exp. Med. **48**: 837, 1928.
3. Hollander, F. and R. Felberg. J. Biol. Chem. **140**: lxii, 1941.
4. Hollander, F. and J. Stein. Federation Proc. **2**: 22, 1943.
5. Hollander, F., J. Stein and F. U. Lauber. Gastroenterology **6**: 576, 1946.
6. Hollander, F. and F. U. Lauber. Proc. Soc. Exp. Biol. Med. **67**: 34, 1948.
7. Ivy, A. C. and Y. Oyama. This Journal **57**: 51, 1921.
8. Lim, R. K. S. and N. M. Dott. Quart. J. Exp. Physiol. **13**: 159, 1923.
9. Loman, J., M. Rinkel and A. Myerson. Am. J. Digestive Diseases **4**: 386, 1937.
10. Myerson, A., M. Rinkel and W. Dameshek. New Engl. J. Med. **215**: 1005, 1936.
11. Takata, M. J. Biochem. (Japan) **2**: 33, 1922.
12. Wright, R. D. and H. W. Florey. Quart. J. Exp. Physiol. **28**: 207, 1938.

FURTHER STUDIES ON XANTHOPTERIN AND OTHER PTERIDINES IN BONE MARROW CULTURES

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In 1936 Osgood and Muscovitz (1) cultured bone marrow in vitro using an elaborate setup, and Osgood and Brownlee (2) devised a simplified procedure for culturing the bone marrow cells. In 1947 Hays (3), using a modification of Osgood's simplified method, studied the reticulocyte production in bone marrow cultures. Plum (4) in 1947 studied the effects of liver preparations on bone marrow cultures using a modification of the original method of Osgood and Muscovitz (1).

Norris and Majnarich (5) have shown that xanthopterin will increase the cell proliferation in bone marrow cultures in vitro, with which a maximum is obtained with 5 γ of xanthopterin per ml. of bone marrow suspension for rat, rabbit, cat, sheep and beef bone marrow. In the experiments previously reported, a suspension of bone marrow cells in tyrodes without glucose was used. The only source of organic matter for the growth and metabolism of the cells would be that introduced with the bone marrow suspension. This would be small and might be a limiting factor in cell proliferation. As it was considered that the conditions used in the previous paper (5) were probably far from optimum, a study was made of some of the factors affecting the rate of cell proliferation in bone marrow cultures.

EXPERIMENTAL

The technique used was that outlined in a previous paper (5), in which 2 ml. of a bone marrow suspension in Tyrode's solution without glucose was placed in a sterilized 5 ml. vial. A glass bead was added to each vial. Supplements were added as indicated in the experiments, and the total volume in the vials was uniform for each experiment. The vial was sealed with a rubber cap and rocked in a water bath at 37°C. Samples for counting the cells were withdrawn at intervals by means of a sterile needle. Beef bone marrow was used in the experiments.

The amino acids were first studies as they might well be a limiting factor in production of new protein in cell proliferation. Figure 1 shows the results obtained for red blood cells (RBC), nucleated cells (NC), and reticulocytes. Combinations of tyrosine, tryptophane, and casein hydrolysate[1] were used. Addition of the various combinations produced a slight increase in the rate of cell proliferation, and all of about the same order of magnitude. Xanthopterin plus the amino acid combinations gave a still further increase in the rate of growth, and all were grouped in the order of magnitude of cell proliferation produced by xanthopterin without the amino acids, with the exception of the combination of casein hydrolysate and tryptophane either with or without tyrosine. Tryptophane would be lacking in the casein hydrolysate produced by acid hydrolysis, and the addition of tryptophane to the hydrolysate would furnish all of the amino acids required for the production of protein.

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[1] The casein hydrolysate used was the 'vitamin-free' sterile 10 per cent solution of acid hydrolyzed casein manufactured by General Biochemicals, Inc., Laboratory Park, Chagrin Falls, Ohio.

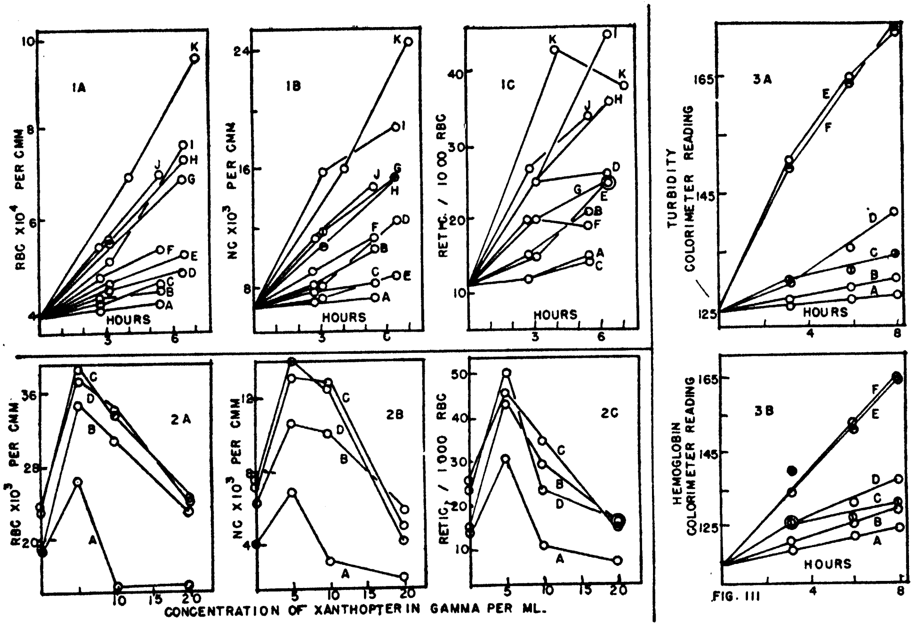

FIG. III

FIG. 1. EFFECT OF AMINO ACIDS AND CASEIN HYDROLYSATE upon the rate of cell proliferation in in vitro bone marrow cultures. The curves have the following supplements per ml. of suspension: *A*, no supplement; *B*, tyrosine, 1 mgm.; *C*, tryptophane, 0.5 mgm.; *D*, casein hydrolysate, 5 mgm.; *E*, casein hydrolysate, 5 mgm., and tryptophane, 0.5 mgm.; *G*, xanthopterin, 0.005 mgm., and tryptophane, 0.5 mgm.; *H*, xanthopterin, 0.005 mgm., and casein hydrolysate, 5 mgm.; *I*, xanthopterin, 0.005 mgm., tryptophane, 0.5 mgm., and tyrosine, 1 mgm.; *J*, xanthopterin, 0.005 mgm., and tyrosine, 1 mgm.; *K*, xanthopterin, 0.005 mgm., casein hydrolysate, 5 mgm., and tryptophane, 0.5 mgm.

FIG. 2. EFFECT OF VARYING CONCENTRATIONS of tryptophane upon the cell proliferation in bone marrow suspensions containing 10 mgm. of casein hydrolysate per ml. of suspension and varying concentrations of xanthopterin. The curves have the following concentrations of tryptophane: *A*, none; *B*, 0.1 mgm. per ml.; *C*, 0.5 mgm.; *D*, 1.0 mgm.

FIG. 3. HEMOGLOBIN FORMATION AND TURBIDITY ASSAY during cell proliferation of bone marrow suspensions. The curves contained the following supplements per ml. of suspension: *A*, none; *B*, xanthopterin, 0.005 mgm.; *C*, xanthopterin, 0.005 mgm., and casein hydrolysate, 5 mgm.; *D*, xanthopterin, 0.005 mgm., and tryptophane, 0.5 mgm.; *E*, xanthopterin, 0.005 mgm., casein hydrolysate, 5 mgm., and tryptophane, 0.5 mgm.; *F*, xanthopterin, 0.005 mgm., casein hydrolysate, 5 mgm., tryptophane, 0.5 mgm., and tyrosine, 1 mgm.

The buffer in the Tyrode's solution was not sufficient to control the pH on the addition of the casein hydrolysate. The hydrolysate had to be neutralized before adding it to the culture and was adjusted to a pH of 7.4 for all tests. However, a final pH varying from 7.0 to 8.0 showed no appreciable variation. The effect of varying the concentration of both casein and tryptophane was studied.

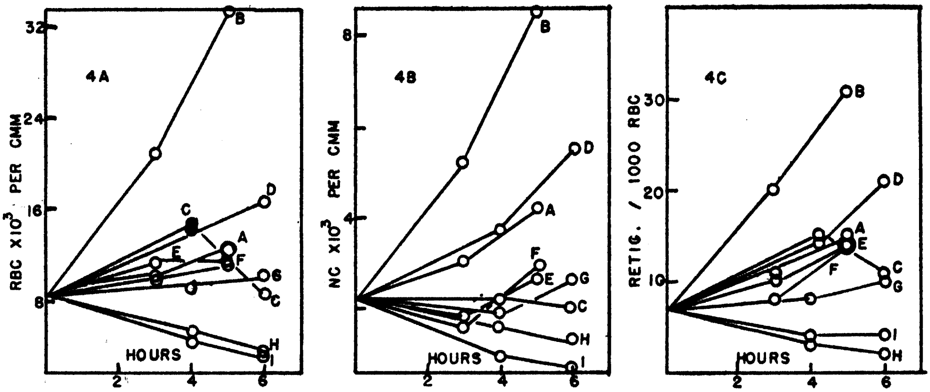

Fig. 4. Effect of various pteridine derivatives on bone marrow cultures in vitro. The curves have the following supplements, each at a concentration of 0.005 mgm. per ml.: *A*, none; *B*, xanthopterin; *C*, isoxanthopterin-6-carboxylic acid; *D*, 6-methyl-isoxanthopterin; *E*, 2-amino-4-hydroxypteridine·HCl·H_2O; *F*, dihydroxanthopterin; *G*, 7-methyl-xanthopterin; *H*, 2-amino-4-hydroxy-7-methyl pteridine; *I*, xanthopterin-7-carboxylic acid.

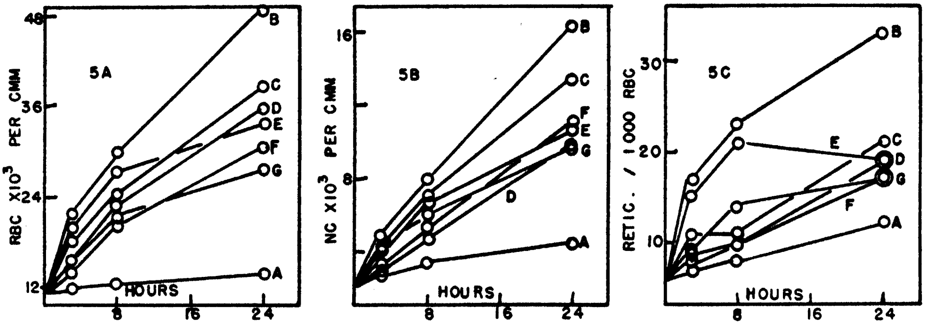

Fig. 5. Effect of various pteridine derivatives on bone marrow cultures in vitro. The curves have the following supplements, each at a concentration of 0.005 mgm. per ml.: *A*, none; *B*, xanthopterin; *C*, 2-amino-4-hydroxy-6-methyl pteridine; *D*, pteroic acid; *E*, 2-amino-4-hydroxy-6-pteridyl methyl pyridinium iodide; *F*, leucopterin; *G*, 2-amino-4-hydroxy-pteridine-6-carboxylic acid.

A concentration of 0.1 ml. of neutralized casein hydrolysate, equivalent to 10 mgm. of casein per ml. of suspension, was selected for all subsequent experiments. Figure 2 shows the response in cell proliferation with varying amounts of tryptophane using the concentration of casein indicated above. From the results a concentration of 0.5 mgm. of tryptophane per ml. of bone marrow suspension was adopted for use. While some variation has been observed between bone marrow preparations, in general the addition of casein hydrolysate and tryptophane not only increases the rate of cell proliferation due to xanthopterin but also broadens the optimum region of concentration of xanthopterin for cell division.

Erythrocyte formation would necessitate hemoglobin formation, which was studied along with the cell counts. A series of test tubes were prepared containing 6 ml. of bone marrow suspension and supplements as indicated in figure 3. A glass bead was added to each test tube, which was sealed with a rubber cap and rocked slowly in a water bath at 37°C. After incubating for intervals of 3, 6 and 8 hours, suspensions were transferred to colorimeter tubes and turbidity read on the Klett-Summerson photoelectric colorimeter. In a uniform suspension of cells the nephelometric reading is indicative of the number of cells. The results are very similar to those indicated by the counts given in figure 1. The cells were then completely laked and hemoglobin determinations made by the alkali method. The results are plotted as colorimeter readings. The increase in hemoglobin is shown to parallel the cell proliferation given in the experiments above and to be greatest when xanthopterin, casein hydrolysate, and tryptophane were added, either with or without added tyrosine.

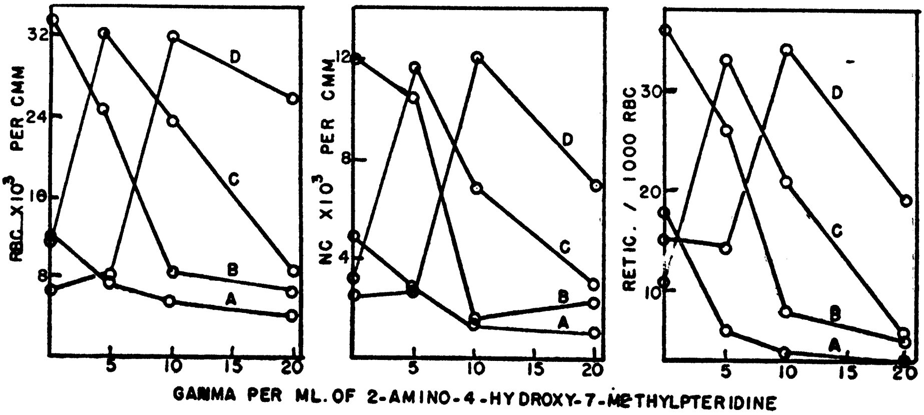

FIG. 6. INTERRELATIONSHIP OF XANTHOPTERIN and 2-amino-4-hydroxy-7-methyl pteridine upon the cell proliferation in bone marrow cultures. The curves represent the following concentrations of xanthopterin per ml. of suspension: *A*, none; *B*, 0.005 mgm.; *C*, 0.010 mgm.; *D*, 0.015 mgm.

The above experiments have shown that xanthopterin increases the rate of cell proliferation in bone marrow cultures in vitro. Several other pteridine derivatives have been tested on in vitro bone marrow cultures and none found with an activity as great as that of xanthopterin. Figures 4 and 5 show representative results. Casein hydrolysate and tryptophane were included in the culture media. 2-amino-4-hydroxypteridine·HCl·H_2O has little or no effect on RBC formation. Those compounds substituted on the six carbon atom stimulate both red and nucleated cell proliferation, such as 2-amino-4-hydroxy-6-methyl pteridine, 2-amino-4-hydroxy-6-pteridyl methyl pyridinium iodide, 2-amino-4-hydroxy-6-carboxylic acid, leucopterin, which is probably converted to xanthopterin, and pteroic acid; however, none of them is as active as xanthopterin. The activity of pteroic acid is of interest compared to pteroylglutamic acid which is entirely inactive. The disubstituted compounds on the 6 and 7 carbon atoms have slight effect on erythrocyte proliferation in vitro and a somewhat greater inhibitory effect upon the nucleated cells. Compounds used were 6 methylisoxanthopterin, isoxanthopterin-6-carboxylic acid, 7 methylxanthopterin, and dihydro-xanthopterin. The two compounds that were distinctly inhibitory and acted as antixanthopterins were 2-amino-4-hydroxy-7-methyl pteridine and xanthopterin-7-carboxylic acid.

The antixanthopterin effect of 2-amino-4-hydroxy-7-methyl pteridine is shown in figure 6. The effect upon cell proliferation appears to be a remarkable balance between xanthopterin and 2-amino-4-hydroxy-7-methyl pteridine. Without added xanthopterin, 2-amino-4-hydroxy-7-methyl pteridine causes a decrease in the number of cells. This inhibitory effect is reversed by adding xanthopterin. We have found repeatedly that if equal concentrations of both are present the rate is not changed materially from what it is with no supplement, but when either is added in excess the effect of the substance in greater concentration becomes evident. The toxic or inhibitory effect of concentrations of xanthopterin greater than 5 γ per ml. is counteracted by added 2-amino-4-hydroxy-7-methyl pteridine. With the addition of increasing amounts of 2-amino-4-hydroxy-7-methyl pteridine the optimum concentration for xanthopterin activity is shifted to such an extent that there would be 5 γ per ml. of xanthopterin in excess in the mixture. That is, for zero 2-amino-4-hydroxy-7-methyl pteridine the optimum is at 5 γ per ml. of xanthopterin; with 5 γ per ml. of 2-amino-4-hydroxy-7-methyl pteridine it is at 10 γ per ml. of xanthopterin and at 10 γ per ml. of 2-amino-4-hydroxy-7-methyl pteridine it is at 15 γ per ml. of xanthopterin. Fifteen γ per ml. of xanthopterin is greater than the optimum concentration and inhibitory but in the presence of 10 γ per ml. of 2-amino-4-hydroxy-7-methyl pteridine gives an activity equal to that of 5 γ per ml. As the molecular weights of the two compounds are very close it appears to be a very surprising stoichiometric relationship between them in control of the cell proliferation.

SUMMARY

1. Amino acids and especially a combination of casein hydrolysate and tryptophane further increase the acceleration of cell proliferation in bone marrow cultures in vitro, produced by xanthopterin.

2. Of several pteridine compounds tested, none showed as great an acceleration of bone marrow proliferation as xanthopterin.

3. Leucopterin, pteroic acid, 2-amino-4-hydroxy-6-methyl pteridine, 2-amino-4-hydroxy-6-pteridyl methyl pyridinium iodide and 2-amino-4-hydroxy-pteridyl-6-carboxylic acid have some activity in stimulating cell proliferation in bone marrow suspensions.

4. Xanthopterin-7-carboxylic acid and 2-amino-4-hydroxy-7-methyl pteridine are strongly inhibitory to cell proliferation in bone marrow cultures.

5. The inhibitory action of 2-amino-4-hydroxy-7-methyl pteridine is counteracted by xanthopterin.
6. The inhibitory action of high concentrations of xanthopterin is counteracted by 2-amino-4-hydroxy-7-methyl pteridine.

The authors wish to gratefully acknowledge and thank the Wellcome Research Laboratories for the generous supply of dihydroxanthopterin, 2-amino-4-hydroxypteridine·$HCl \cdot H_2O$, 6-methyl-isoxanthopterin, 7-methyl xanthopterin, isoxanthopterin-6-carboxylic acid and xanthopterin-7-carboxylic acid used in our experiments.

The authors wish to gratefully acknowledge and thank the Lederle Laboratories, Division of the American Cyanamid Company, for the generous supply of 2-amino-4-hydroxy-6-methyl peteridine, 2-amino-4-hydroxy-7-methyl pteridine, 2-amino-4-hydroxypteridine-6-carboxylic acid and 2-amino-4-hydroxy-6-petridyl methyl pyridinium iodide used in our experiments.

The authors wish to gratefully acknowledge and thank Merck and Co., Inc. for the generous supply of pteroic acid used in our experiments.

REFERENCES

1. Osgood, E. E., and A. N. Muscovitz. J. Am. Med. Assoc. **106**: 1888, 1936.
2. Osgood, E. E. and I. S. Brownlee. J. Am. Med. Assoc. **107**: 123, 1936.
3. Hays, E. E., Proc. Soc. Exp. Biol. Med. **63**: 558, 1946.
4. Plum, C. M., J. Hematol. Special Issue No. 1, p. 33, 1947.
5. Norris, E. R. and J. J. Majnarich. This Journal. In press.

REDUCTION OF CENTRAL HYPER-IRRITABILITY FOLLOWING BLOCK ANESTHESIA OF PERIPHERAL NERVE

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The purpose of this study was to determine whether hyper-irritability of the dental innervation, produced by previous painful stimulation of the teeth, could be permanently reduced to a normal level of irritability by a single application of procaine block.

This question arose from two previous studies (1, 2). In the first (1) it was observed that the most prevalent cause of dental pain engendered by alteration of barometric pressure (aerodontalgia) was referred pain from an occluded paranasal (probably maxillary) sinus. Not only was this pain referred to the teeth, but in an overwhelming majority of cases, it was referred to teeth which showed evidence of previous painful stimulation as by filling, trauma, caries or extraction.

This led to the hypothesis that the mechanism involved in the referral of pain in this case was one akin to spatial summation of afferent stimuli in the motor reflex and that the stimulus arising from the sinus only 'facilitated' a chronic sub-threshold stimulus from the offending tooth. Since the injury to the teeth in this study was in many cases made years previous to the observation, the authors were intrigued by this evidence of a prolonged 'central excitatory state'. Therefore, a second study (2) was set up to determine whether this observation could be repeated under conditions whereby all known factors could be controlled. Fillings and extractions were made without block anesthesia (NO was used for extractions) and after an interval of several weeks the nasal epithelium was stimulated mechanically, by a needle prick, in the region of the maxillary sinus ostium. With a single exception, pain was referred to the previously stimulated teeth whether or not any sensation was evoked from the site of the immediate stimulation.

Collateral observations made in the above studies indicated that hyperesthesia does not exist in teeth receiving dental treatment under the influence of procaine block anesthesia. This study was set up to determine whether procaine block is efficacious in removing an already established hyperesthesia.

SUBJECTS AND METHODS

The subjects reported on in this paper were the same as those used in a previous investigation (2). These had undergone dental treatment of approximately bilateral symmetry either without anesthesia, or under general nitrous oxide anesthesia. Following this treatment all subjects exhibited a tendency for occurrence of pain in the treated teeth elicited by mechanical stimulation of the ipsolateral maxillary ostium.

Since the subjects had received approximately equivalent treatment bilaterally, it was decided that the upper and lower quadrants of the dextral dentition could be used to test the efficacy of procaine anesthesia in eliminating this susceptibility to referral of pain, while the sinistral side could be left unanesthetized as a control.

In order to determine whether elevated irritability was still existent, the right maxillary ostium was mechanically stimulated, by means of a needle prick, and the occurrence of pain as reported by the subject recorded. Following this procedure, block anesthesia of the dentition in the area to which pain was referred was produced, using an injection of two per cent solution of procaine hydrochloride with epinephrine in a concentration of one part in 50,000.

The locations of the blocks were as follows:

1. For the maxillary dentition, infraorbital (anterior and middle superior alveolar branches of the maxillary division of the trigeminal nerve); infratemporal (posterior superior alveolar branches of the maxillary division of the trigeminal nerve); greater palatine and nasopalatine blocks were used.
2. For the mandibular teeth, the mandibular or inferior alveolar block was used (lingual nerve before it enters the region of the floor of the mouth and the inferior alveolar before it enters the mandibular canal at the lingula). This was supplemented by blocking the buccinator nerve.

Approximately two weeks (10 to 16 days) after production of the temporary block, both maxillary ostia were stimulated and the incidence of tooth pain recorded.

The results of stimulation of the maxillary sinus ostia are given in table 1.

From table 1 it may be seen that mechanical stimulation of the maxillary sinus ostium was effective in eliciting tooth pain in dextral teeth in 100 per cent of the cases before anesthesia.

Approximately two weeks (10 to 16 days) after use of procaine block anesthesia, no referred pain to dextral teeth could be produced by ipsolateral maxillary ostium stimulation. The 'control' sinistral teeth still responded with strong pain sensation upon stimulation of the ipsolateral maxillary ostium in 13 of 14 subjects (93 per cent of the cases).

DISCUSSION

From a standpoint of practical dentistry, the observations reported present a simple method of alleviating tooth pain referred from the maxillary sinus.

Of equal importance from a fundamental standpoint are the implications of these results with respect to the neurophysiology of pain mechanisms. The authors are very fortunate in this instance in having available an example of 'pain facilitation' in which both sources of stimulation are known and capable of manipulation. Denslow and his co-workers (3, 4) have reported a closely allied condition in the case of pain perception from pressure on the spinous processes of the thoracic vertebrae. In their work a considerable advantage is reported from the presence of motor activity associated with pain transmission. This allows collection of objective evidence of neural activity which is much more

TABLE 1. EXPERIMENTAL DATA

SUBJECT		DENTAL WORK[1]		INTERVAL 1	DENTAL PAIN	ANESTHESIA	INTERVAL 2	DENTAL PAIN	INTERVAL 3
Sex	Age	Tooth	Proc	Days	Stim-1	Used	Days	Stim-2	Days
M	23	3	11	49	+	+	13	−	62
		14	1			−		+	62
M	14	4	1	53	+	+	12	−	65
		14	1			−		+	65
M	52	3	1	55	+	+	11	−	66
		14	11			−		+	66
M	28	2	1	52	+	+	11	−	63
		13	11			−		+	63
M	21	3	11	30	+	+	11	−	41
		14	1			−		−	41
M	24	4	11	50	+	+	13	−	63
		14	11			−		+	63
M	22	3	11	46	+	+	11	−	56
		13, 14	11, 1			−		+	56
M	22	3	11	59	+	+	10	−	69
		13	1			−		+	69
F	28	2, 3	11, 1	53	+	+	15	−	68
		15	1			−		+	68
F	23	3	11	50	+	+	16	−	66
		14	1			−		+	66
F	22	4	V	53	+	+	16	−	70
		13	11			−		+	70
M	30	3	11	53	+	+	14	−	67
		13, 14	1, 1			−		+	67
M	27	20	11	38	+	+	16	−	54
		30, 31	1, 1			−		+	54
F	28	3	Ext.	37	+	+	11	−	48
		13, 14, 15	Ext.			−		+	48

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[1] Fillings according to Black's (1936) classification: I—occlusal surface, II—mesio or disto-occlusal surfaces; V—gingival surface; Ext.—extraction.

Interval 1. Days elapsing between original dental treatment and stimulation of maxillary sinus ostium just prior to procaine injection.

Interval 2. Days elapsing between procaine injection and final stimulation of maxillary sinus ostium.

Interval 3. Total time (days) elapsing between original dental treatment and final maxillary stimulation.

satisfactory in many respects than the purely subjective responses upon which the present paper is based. Nevertheless, the authors believe that subjective evidence of positive character, such as the dental pain used as the criterion for this study, allows a high degree of confidence in the results.

Previous work by Hardy *et al.* (5), Denslow, (3, 4), Livingston (6), Heinbecker (7) and the present authors (1, 2) has indicated that referred pain may be due to facilitation of a sensory pathway maintained at an elevated level of irritability, by stimulation of another sensory pathway in the same segment. Although this observation is fairly well established (5), there has been relatively little insight into the mechanism by which the elevated central irritability is maintained. Some workers, Sherrington (8) and Livingston (6), have suggested a 'vicious circle' concept involving afferent autonomic pathways which maintain the tissue in the region of the pain receptor in a disturbed physiologic state. Some evidence for this is found in the cases of 'phantom limb pain', causalgia and other similar conditions. Denslow reported a 'doughy consistency' of the tissue in the area of vertebral spinous processes with low pain thresholds.

In the present work, two possibilities for maintenance of elevated irritability are manifest. The simplest case would be that of a disturbed physiologic condition of the dental pulp due directly to the presence of filling materials, anatomical changes, etc. in the immediate vicinity. Another possibility is that of a disturbed circulation to the dental pulp resulting from autonomic activity in concurrence with the 'vicious circle' concept. The observations reported in the present paper indicate that the latter is the more likely explanation since reduction of the elevated irritability is effective for as long as 16 days when accomplished by a single anesthetic block of the innervation. If the elevated irritability were due to reactions continuously produced by irritating agents or structures in the dentition, one would expect that symptomology would return immediately on recovery from anesthesia.

Further, it appears from the foregoing observations that the receptor neuron itself is an important factor in maintaining an elevated central irritability. This does not discount, however, the possible importance of the autonomic innervation in maintaining a localized tissue disturbance at the site of the receptor endings.

CONCLUSIONS

1. An elevated central irritability is maintained in the sensory pathway in teeth undergoing dental treatment without local anesthesia for a long period (two months in the present paper, but previous work indicates that this may exist for several years).
2. The elevated central irritability may be reduced for an extended period by temporary procaine block anesthesia of the innervation.
3. Since referred pain to the teeth from the maxillary sinus depends upon elevated irritability of the dental sensory pathway, this procedure eliminates further dental pain of this origin either permanently or for a considerable period of time.
4. The afferent peripheral neuron itself is an important unit in the maintenance of elevated central irritability.

REFERENCES

1. Reynolds, O. E., H. C. Hutchins, A. Y. Werner, and F. R. Philbrook. Naval Med. Bull. **46:** 845, 1946.
2. Hutchins, H. C. and O. E. Reynolds. Journal of Dental Research **26:** 3, 1947.
3. Denslow, J. S. and C. C. Hassett. J. Neurophysiol. **5:** 393, 1942.
4. Denslow, J. S. J. Neurophysiol. **7:** 207, 1944.
5. Hardy, J. D., H. G. Wolff, and H. Goodell. J. Clin. Invest. **19:** 649, 1940.
6. Livingstone, W. K. Pain Mechanisms. New York, The Macmillan Co., 1943. P. 253.
7. Heinbecker, P. and G. H. Bishop. Sensation: Its Mechanisms and Disturbances. Baltimore, Williams & Wilkins Co., 1935. Chapter 9.
8. Sherrington, C. S. The Selected Writings of Sir Charles Sherrington. New York, Paul B. Hoeber, 1940. Pp. XIV, 532.

RATE OF RECOVERY OF TEMPERATURE-REGULATING RESPONSES AFTER ETHER ANESTHESIA

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All investigators who have studied the effect of anesthesia on temperature regulation of warm-blooded animals have found that anesthetics depress temperature-regulating functions. In previous work (1) it was found that morphine so disturbed temperatue regulation that dogs under this anesthetic would shiver and pant simultaneously. Animals anesthetized with barbiturates, chlorolosane or morphine (2, 3) are unable to maintain rectal temperatures which fall one to five degrees below normal values. This inability to maintain a normal body temperature has been shown to be due to *a*) a failure of peripheral vasoconstriction in response to cold (1, 4) and *b*) a failure of the shivering mechanism to respond to a fall in rectal temperature (1).

The depressing action of anesthetics on the finer regulation of body temperature has required that physiologists studying this problem use non-anesthetized trained animals. This imposes serious limitations on many experimental methods. Where minor surgical procedures are involved, such as arterial and venous catheterization or the insertion of thermocouples in deeper structures, a possible compromise to this difficulty could consist of performing the minor surgery under a gaseous anesthetic and then allowing the animal several hours to eliminate the anesthetic after which the normal regulation of body temperature might be regained. Gaseous anesthetics are desirable due to their rapid elimination and due to the rapid recovery of the animal after discontinuing anesthesia. For these reasons a study has been made of the rate at which dogs recover their ability to regulate body temperature after thirty minutes of light ether anesthesia. The criterion for recovery of normal temperature regulation consisted of a standardized cold exposure test in which the following temperature-regulating responses were measured: *a*) ability to maintain normal body temperature, *b*) ability to produce peripheral vasoconstriction on exposure to cold and *c*) ability to shiver without an abnormal drop in rectal temperature.

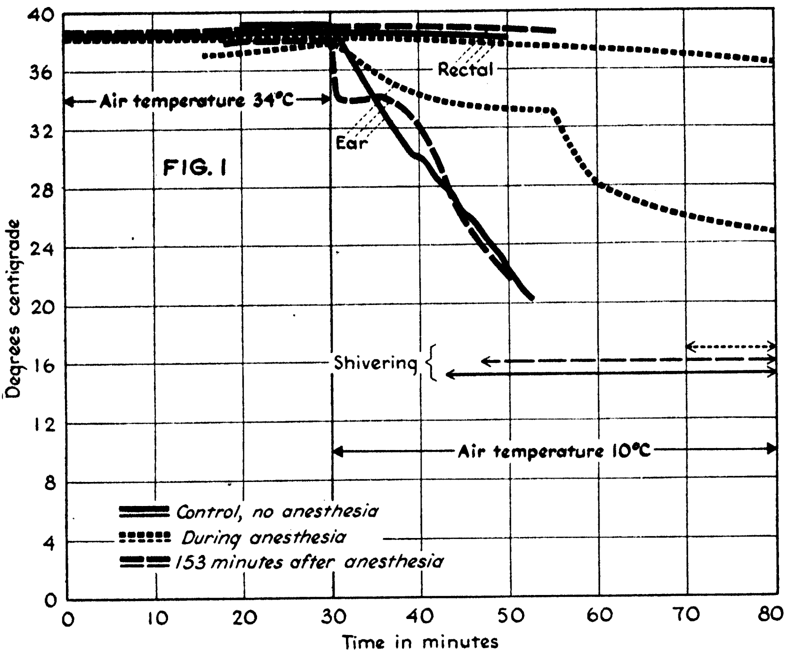
EXPERIMENTAL

Medium weight short-haired dogs were used. The animals were selected and trained to lie quietly on a table beneath a wooden box cover which was joined by hinges to the table top. The cold exposure test was made in a room at 9° to 10° C. with an air velocity of 25 to 50 feet/minute and relative humidity of 50 to 75 per cent. When closed the interior of the box in which the dog was lying was maintained at an air temperature of 34° C. by means of electrical heaters. After 30 minutes of resting in this warm environment the box heaters were turned off and the box swung open on its hinge exposing the dog suddenly to the cold environment of 10° C. During this abrupt change of environmental temperature the animal did not move but remained lying on its side in a resting position on the table top. Skin and rectal temperatures were measured throughout the test in both warm and cold environments. Exposure to cold was continued until shivering was severe as estimated by visual inspection.

The skin temperatures were measured by thermocouple junctions of 36-gauge copper and constantin wire attached to a small shave area of skin with adhesive tape ¼"x 1". Skin temperatures were measured on the foreleg over the lower anterior surface of the tibia, the side of the thorax and the ear. As in previous work (5) it was found that the ear temperature change was the most striking evidence of peripheral vasoconstriction. A record of the changes of ear temperature and rectal temperature during cold exposure tests is given in figure 1 which includes three tests on the same dog (*dog F*): *a*) control (no anesthesia), *b*) during ether anesthesia and *c*) when exposure to cold, i.e., opening of the box cover, was made 153 minutes after discontinuing ether anesthesia. The onset of shivering after exposure to cold is indicated by the blocks of the diagram in the lower right of the figure.

Ether-air mixtures were prepared in two 100-liter spirometers by adding from a burette a known volume of liquid ether to a heated pressure bottle through which a measured volume of air was drawn into the spirometer. One spirometer was being filled while the air-ether mixture in the other was being breathed by the dog. A sample of the air-ether mixture was drawn from the hose leading to the tracheal cannula and analyzed for ether content. The animal was made unconscious by administration of ether by the ether cone, a procedure lasting one to two minutes. As soon as possible the cone was removed and a tracheal cannula inserted through the mouth and the prepared ether mixture was inhaled. The ether content of the gaseous mixture breathed was 10 to 11 mgm. per 100 ml. of mixture. This resulted in a blood ether concentration which varied between 100 to 120 mgm. of ether per 100 ml. of whole arterial blood. According to Beecher (6) light ether anesthesia is maintained with a blood ether concentration of 100 mgm/100 ml. of blood while Robbins (7) gives as the average anesthetic concentration of ether 115/mgm/100 ml. of blood and states that a concentration of 100 to 110 mgm/100 ml. blood is sufficient for anesthesia. The state of anesthesia observed in the experiments was estimated as being 'light' and adequate for minor surgery. Ether anesthesia was continued for 30 minutes. After discontinuing ether anesthesia the animal was allowed to recover at room temperature of 23 to 25° C. for variable lengths of time in different experiments and was placed in the warm environment at 34° C. 30 minutes before exposure to cold. In each experiment the following routine was observed:

1. *Period of anesthesia.* This period lasted 30 minutes in all experiments except those in which tests were made during anesthesia. In this latter case ether anesthesia was continued throughout all the periods. Room temperature was 23–25° C.



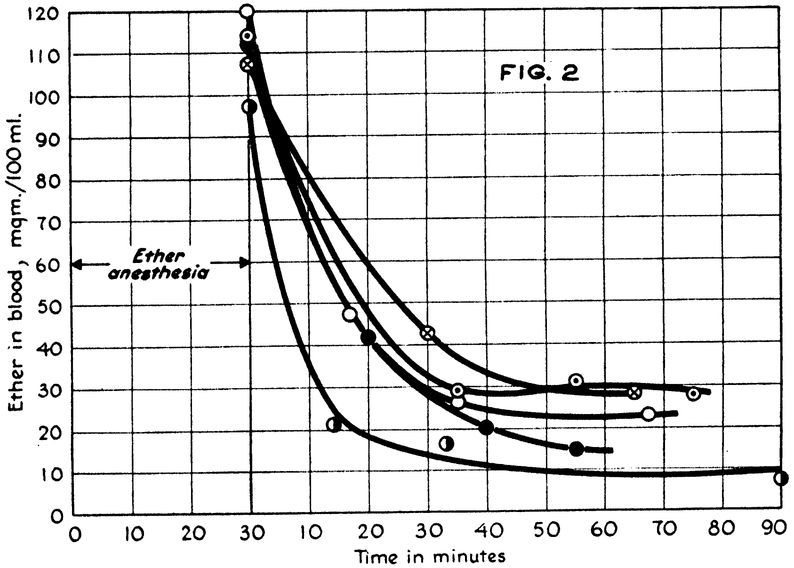


Fig. 1. Rectal and ear temperatures during exposure to a warm environment of 34°C. followed by exposure to a cold environment of 10°C. Records of three experiments: *a*) control experiment, (no anesthesia), *b*) during ether anesthesia and *c*) exposure to cold 153 minutes after cessation of ether anesthesia.

Fig. 2. Rate of elimination of ether from the blood after discontinuing light ether anesthesia. Five dogs were used.

2. *Recovery period.* The animal was allowed to recover from the anesthetic in an environment of 23 to 25° C. This period lasted from 0 to 150 minutes.

3. *Warming period.* The dog was rested for 30 minutes in a box at 34° ± 1° C. in order to produce a standard thermal state.
4. *Exposure period.* The hinged box was opened and the resting animal without being disturbed was exposed to an environment of 10° ± 1° C. The experiment terminated when shivering became violent.

Ether in air and blood was measured by the usual Nicloux oxidation method in which the ether, after distillation, is oxidized to acetic acid by a known amount of chromic acid and the excess chromic acid remaining unreduced is determined by titration. For measuring ether in blood, flasks of the Widmark (8) and Cavett (9) type were used. Recent investigators have used methyl orange as an indicator for the titration of excess chromic acid in ether and alcohol determinations. This indicator was found to give a poorly defined end point. Considerable improvement in the sharpness of the titration end point was achieved by using barium diphenylamine sulphonate in the presence of phosphoric acid, Kolthoff (10). Details of this method have been recently described (11).

RESULTS

The rate at which ether is eliminated from the blood of five dogs after 30 minutes of light ether anesthesia with blood levels of 100 to 120 mgm. ether per 100 ml. of whole blood is represented by the curves of figure 2. Ether is rapidly eliminated during the first 30 minutes after discontinuing ether anesthesia. Following this period of rapid elimination the rate decreases slowly with values of 10 to 30 mgm. ether per 100 ml. of blood being found one to two hours after anesthesia.

Data from a cold exposure test are given in figure 1. After resting for 30 minutes in a warm environment at 34° C. the animal without moving is exposed to an environment of 10° C. Only ear and rectal temperatures for one dog are given in figure 1. It will be noted that on exposure to cold there is a sudden drop of ear temperature. For the control animal the ear temperature decreased rapidly to 34° where it remained for 4 minutes and then rapidly fell to less than 20° C. The initial decrease in temperature is probably due to cooling of the skin thermocouple by the exposure to cold air where the skin temperature falls because of the cold environment. The second drop in temperature can be interpreted as being due to peripheral vasoconstriction. As an arbitrary index vasoconstriction is assumed to be in active progress when the ear temperature has reached 30° C. It will be noted in figure 1 that with a normal unanesthetized (control) animal vasoconstriction occurred after a cold stimulus of 12 minutes. With the same animal exposed to cold 153 minutes after cessation of ether anesthesia vasoconstriction occurred after a cold stimulus of 12 minutes. When the same animal was maintained under anesthesia and exposed to cold vasoconstriction (i.e., the ear temperature reached 30° C.) occurred after 28 minutes of exposure to cold.

When temperature regulatory mechanisms are depressed by anesthetics the rectal temperature of a dog at which shivering starts is lowered below control non-anesthetized values (2). Hence, one criterion of the depressant effect of anesthesia is the rectal temperature threshold at which shivering starts. These values are given in table 1 for *a*) control (no anesthesia) dogs, *b*) dogs exposed to cold during anesthesia and *c*) dogs exposed to cold at varying time intervals after cessation of anesthesia. It will be noted that during anesthesia and in the first hour following cessation of anesthesia the rectal threshold values for shivering were subnormal while values two to three hours after anesthesia were normal.

It was noted in the earlier experiments on the effect of nembutal on temperature regulation (2) that the anesthetic prolongs the exposure time to cold before shivering starts. In the experiments reported here on ether anesthesia the exposure time to cold, required to produce shivering in unanesthetized, anesthetized and post-anesthesia dogs, was measured. The results are given in table 1. The 'exposure time' values are the time intervals between the opening of the box exposing the dog to the cold and the beginning of shivering. It will be observed that the exposure times were quite variable for controls as well as for the etherized animals. Except for two experiments, namely *dog A* exposed to cold 59 minutes after anesthesia and *dog C* exposed 116 minutes after anesthesia, shivering occurred during and after ether anesthesia almost as promptly as with the controls. There was a difference in the individual response of dogs. *Dog B* shivered in all experiments after 0 to 8 minutes' exposure to cold while *dog E* required 3 to 34 minutes of the cold stimulus. *Dog C* was a short-haired dog of the terrier type while *dog E* has somewhat longer and curly hair, apparently being partially of the setter type. The slow onset of shivering in *dog E* might be attributed to a difference in the length of the hair.

The test which is of most significance in evaluating the depressive action of anesthesia on physiological temperature regulation is a test of the ability to maintain rectal temperature after exposure to cold. Data from 31 cold exposure tests are given in table 2. The change in rectal temperature during a 30-minute period in the cold is given in the column 'ΔTR' of table 2. TR is the value of the rectal temperature at the end of the rest period in the warm environment when the box was lifted and the dog was exposed to cold. The rectal temperature 30 minutes later was subtracted from TR to give ΔTR. Normal control dogs when resting in a cold environment of 10° C., after changing from a warm environment of 34° C., will usually have a reduction of rectal temperature varying from 0 to 0.4° C. There are significant individual differences in animals as is seen in table 2. *Dog B* usually exhibited a rise in rectal temperature after exposure to cold, the increase usually being 0.1 to 0.2° C. In striking contrast, the rectal temperature of *dog E* usually fell 0.3 to 0.5° C. under the same conditions of testing. When the rectal temperature measurements were made during ether anesthesia the two animals tested under these conditions had rectal temperature decreases of 0.8 and 1.5° C. However, two hours after ether anesthesia the decreases of rectal temperature, resulting from a 30-minute exposure to cold, were normal.

It had been found previously (5) that when dogs were exposed to cold the first response was a peripheral vasoconstriction followed secondarily by shivering. In the present experiments this procedure occurred frequently but in many cases shivering preceded or was almost simultaneous with peripheral vasoconstriction. It was also found that the longer the experiments were continued the greater was the tendency for shivering to precede peripheral vasoconstriction. The animals were trained and had been tested many times in the same environment and it appeared that a state of 'conditioned shivering' was developing resembling the 'conditioned panting' to a warm environment described by Sinelnikoff (12). In other words the animals which were frequently subjected to the testing routine became conditioned and anticipated the cold by early shivering. This early

TABLE 1. THRESHOLD RECTAL TEMPERATURES AT WHICH SHIVERING STARTED AND EXPOSURE TIME TO A COLD ENVIRONMENT REQUIRED FOR INITIATION OF SHIVERING

DOG	EXPERIMENT NUMBER	RECTAL TEMPERATURE	EXPOSURE TIME	CONDITION
		°C.	*min.*	
A	1	39.6	2	Control, no anesthesia
	2	39.4	5	" " "
	3	39.4	8	" " "
	4	38.0	13	During anesthesia
	5	38.5	84	59 minutes after anesthesia
	6	39.2	8	60 minutes after anesthesia
	7	39.2	22	125 minutes after anesthesia
	8	39.0	13	128 minutes after anesthesia
B	1	38.6	7	Control, no anesthesia
	2	38.4	4	" " "
	3	38.3	2	" " "
	4	38.7	2	" " "
	5	37.8	0	30 minutes after anesthesia
	6	38.5	3	67 " " "
	7	38.2	0	70 " " "
	8	38.5	3	95 " " "
	9	38.4	8	125 " " "
	10	39.0	7	180 " " "
D	1	39.1	15	Control, no anesthesia
	2	38.7	11	" " "
	3	38.8	7	" " "
	4	38.1	8	78 minutes after anesthesia
	5	38.2	17	116 " " "
	6	39.1	7	120 " " "
	7	39.0	10	190 " " "
E	1	38.8	3	Control no anesthesia
	2	38.7	13	" " "
	3	38.4	34	" " "
	4	37.7	23	During anesthesia
	5	38.1	23	100 minutes after anesthesia
	6	38.4	27	153 " " "

shivering apparently produced sufficient metabolic heat to delay the onset of peripheral vasoconstriction. From a study of many skin temperature records it has been arbitrarily decided that vasoconstriction is in active progress in the testing routine described when the falling ear temperature reached 30° C. (see fig. 1). In many tests shivering was so pronounced that the ear temperature

TABLE 2. Rectal temperatures (TR) before exposure to cold and change in rectal temperature (ΔTR) caused by 30 minutes exposure to cold. Exposure time in cold before ear temperature reached 30° C.

DOG	EXPERIMENT NUMBER	TR	ΔTR	EXPOSURE TIME	CONDITION
		°C.	°C.	min.	
A	1	39.6	−0.1	—	Control no anesthesia
	2	39.4	−0.2	—	“ “ “
	3	39.5	−0.2	—	“ “ “
	4	39.0	−1.5	37	During anesthesia
	5	39.4	−0.4	8	59 minutes after anesthesia
	6	39.3	−0.3	—	60 “ “ “
	7	39.8[1]	−0.7[1]	—	125 “ “ “
	8	39.2	−0.2	27	127 “ “ “
B	1	38.5		10	Control no anesthesia
	2	38.3		—	“ “ “
	3	38.2	+0.1	—	“ “ “
	4	38.6	+0.2	—	“ “ “
	5	37.8	+0.1	—	30 minutes after anesthesia
	6	38.3	+0.2	—	67 “ “
	7	38.2	[2]	—	70 “ “ “
	8	38.4	+0.1	—	95 “ “ “
	9	38.2	[3]	5	125 “ “ “
	10	38.8	+0.3	5	180 “ “ “
C	1	39.4	−0.2	—	Control, no anesthesia
	2	39.0	−0.4	24	“ “ “
	3	38.8	−0.3	45	“ “ “
	4	39.2	[3]		78 minutes after anesthesia
	5	38.6	−0.4	25	116 “ “ “
	6	39.0	0.0	42	120 “ “ “
	7	39.2	−0.3	—	190 “ “ “
D	1	39.0	−0.4	15	Control, no anesthesia
	2	39.0	−0.5	14	“ “ “
	3	39.0	−0.5	21	“ “ “
	4	38.4	−0.8	28	During ether anesthesia
	5	38.8	−0.7	28	100 minutes after anesthesia
	6	38.6	−0.3	10	

[1] Warm cabinet had become overheated reaching 38° C. instead of 34° C.

[2] Rectal thermometer slipped from rectum.

[3] Experiment terminated before 30 minutes of cooling.

remained elevated for longer than the 30-minute cold period. In table 2 are given the exposure times to cold before peripheral vasoconstriction was in active progress as indicated by a fall in ear temperature of 30° C. in the cold environment of 30° C. It will be noted that peripheral vasoconstriction did occur during ether anesthesia and in the three hours following ether anesthesia. Compared with the normal controls the ether anesthesia did not prolong significantly the time required for peripheral vasoconstriction. In deep anesthesia with other anesthetics, for example, barbiturates (2), anesthesia abolishes peripheral vasoconstriction.

DISCUSSION

These results indicate that ether anesthesia in dosages necessary for light surgical anesthesia has a depressing effect on temperature-regulating responses. Neither shivering nor peripheral vasoconstriction are abolished but the fine regulation of these responses is sufficiently impaired that rectal temperature cannot be maintained. After discontinuing ether anesthesia recovery is rapid and the normal thermoregulatory state for maintenance of body temperatue in the cold is reached within two to three hours after cessation of ether breathing.

SUMMARY

Medium-sized dogs were subjected to a cold exposure test for determining the thermoregulatory responses of *a*) shivering, *b*) peripheral vasoconstriction due to cold and *c*) maintenance of rectal temperature. The test was performed on unanesthetized dogs, dogs under anesthesia and the same animals at varying times after anesthesia. Neither shivering nor peripheral vasoconstriction are abolished by the anesthetic but fine regulation of body temperature is impaired to the degree that normal rectal temperatures cannot be maintained in the cold. The temperature regulatory responses of dogs are normal within two to three hours after cessation of ether anesthesia.

REFERENCES

1. Hemingway, A. J. Pharm. Exp. Therap. **63**: 414, 1938.
2. Hemingway, A. This Journal **134**: 350, 1941.
3. Green, H. D., N. D. Nickerson, R. N. Lewis and B. L. Brofman. This Journal **140**: 177, 1943.
4. Morton, J. J. and W. J. M. Scott. J. Clin. Inv. **9**: 235, 1930.
5. Hemingway, A. This Journal **128**: 736, 1940.
6. Beecher, H. K. The Physiology of Anesthesia. New York, Oxford University Press, 1938.
7. Robbins, B. H. Anes. and Analgesia **15**: 183, 1936.
8. Widmark, E. M. P. Biochem. Zs. **131**: 473, 1922.
9. Cavett, J. W: J. Lab. Clin. Med. **23**: 543, 1938.
10. Kolthoff, I. M. and E. B. Sandell. Textbook of Quantitative Inorganic Analysis. New York, MacMillan Co., 1938. P. 472.
11. Hemingway, A., L. A. Bernat and J. C. Maschmeyer. J. Lab. Clin. Med. **33**: 126, 1948.
12. Sinelnikoff. Pfluegers Arch. **221**: 548, 1928–29.

POSTURAL CHANGES IN VITAL CAPACITY WITH DIFFERENTIAL CUFF PRESSURES AT THE BASES OF THE EXTREMITIES[1]

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The reduction in vital capacity coincident with recumbency was described by Hutchinson (1). Hamilton and Morgan (2) found that pooling of blood in the extremities produced by inflating blood pressure cuffs to diastolic level resulted in an increase in the recumbent vital capacity. Dow (3) found that in 42 normal subjects the vital capacity decreased 192 cc. on lying supine; but when pooling of blood in the four extremities was produced, the recumbent vital capacity recovered 102 cc. However, the above investigation actually produced progressive pooling of blood in the arms and legs by inflating cuffs only to diastolic level. It is the purpose of this paper to report the results obtained with blood pressure cuffs inflated to a pressure above systolic level in order to maintain the volume of blood in the extremities at a constant level.

Nine young adult male subjects were used in these studies of vital capacity in the standing and recumbent position with cuffs at the bases of the extremities inflated to diastolic pressure, above systolic pressure, and with the cuffs non-inflated. Equipment included an ordinary spirometer and a precision wet test gas meter. A pressure reservoir was utilized so that simultaneous filling of the B.P. cuffs was possible. At least 10 readings of vital capacity were taken in the various conditions for each subject.

RESULTS

Table 1 below shows that in group I there is 185 cc. mean reduction in vital capacity when a standing subject with non-inflated cuffs assumes the supine position. The latter condition is the only one in this group with a significant deviation from column 1. In column 5 the cuff pressure was raised to above systolic level just before the standing subject would recline, thus occluding arterial inflow into, as well as venous return from, the extremities. There is an insignificant 6 cc. discrepancy between columns 1 and 5, showing that the reflux of venous blood from the trunk and head did not significantly lower vital capacity in these subjects. A comparison of the first four columns reveals that cuffs maintained at diastolic level nullify the decrease in recumbent vital capacity found in the same subjects with non-inflated cuffs.

It was noted that an intense reactive hyperemia, as described by Lewis (4), follows the release of systolic pressure cuffs. In addition, bradycardia and hypotension regularly occurred with the subjects in the erect posture. Syncope was manifest in two of the five subjects studied. In the recumbent position the

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[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

bradycardia and hypotension did not occur although the same degree of reactive hyperemia was present. Observations on vital capacity in the erect posture with systolic cuffs cannot be repeated in close succession without obtaining fictitiously low values, due presumably to the above effect.

With this pitfall in mind, group II (table 2) was studied with reference to comparing vital capacity standing with non-inflated cuffs with vital capacity standing and supine with cuffs inflated to above systolic blood pressure. There is no significant difference in the vital capacities of subjects standing with non-inflated cuffs as opposed to standing with cuffs at above systolic pressure.

TABLE 1

GROUP I[1]

	STANDING WITH DEFLATED CUFFS 1	SUPINE WITH DEFLATED CUFFS 2	STANDING WITH DIASTOLIC CUFFS 3	SUPINE WITH DIASTOLIC CUFFS 4	SUPINE WITH SYSTOLIC CUFFS 5
C. S. B.	5035 ±16 P.E.	4837 ±30 P.E.	4991 ±8 P.E.	4889 ±22 P.E.	4836 ±33 P.E.
G. S. C.	4809 ±12 P.E.	4625 ±18 P.E.	4802 ±14 P.E.	4889 ±14 P.E.	4938 ±14 P.E.
K. W. C.	5302 ±16 P.E.	5183 ±15 P.E.	5289 ±13 P.E.	5258 ±11 P.E.	5266 ±14 P.E.
W. L. W.	5947 ±11 P.E.	5590 ±10 P.E.	6041 ±13 P.E.	5944 ±15 P.E.	6043 ±14 P.E.
D. J. S.	5181 ±9 P.E.	5112 ±11 P.E.	5078 ±13 P.E.	5155 ±11 P.E.	5165 ±9 P.E.
Mean	5254	5069	5240	5227	5249

[1] Vital capacity expressed in cc.

TABLE 2

GROUP II[1]

	STANDING WITH DEFLATED CUFFS 6	STANDING WITH SYSTOLIC CUFFS 7	SUPINE WITH SYSTOLIC CUFFS 8
R. B. H.	4966 ±16 P.E.	5074 ±17 P.E.	4797 ±15 P.E.
C. R. H.	4014 ±7 P.E.	3986 ±13 P.E.	3776 ±9 P.E.
J. P. L.	5240 ±24 P.E.	5183 ±18 P.E.	4826 ±15 P.E.
H. L. H.	4736 ±17 P.E.	4788 ±9 P.E.	4595 ±12 P.E.
Mean	4746	4757	4498

[1] Vital capacity expressed in cc.

The subjects in group II had the cuffs raised to above systolic blood pressure after reclining for 3–5 minutes (in contrast to group I whose cuffs were inflated before the subjects reclined). Consequently column 8 displays a significant 248 cc. reduction in vital capacity when compared to column 6, indicating that the pooling of blood in the lungs of a recumbent individual will in less than 3–5 minutes result in a decreased vital capacity.

CONCLUSION

1. The vital capacities were determined in nine adult male subjects in the standing and supine positions with cuffs placed at the bases of the arms and legs

inflated to diastolic pressure, above systolic pressure, and non-inflated. When the cuffs were inflated to a level above systolic blood pressure before the standing subjects would recline, the reflux of venous blood from the trunk and head did not lower vital capacity.

2. There was no difference between the vital capacity of subjects standing with the cuffs inflated above systolic pressure and the vital capacity of these same subjects standing with non-inflated cuffs.

REFERENCES

1. HUTCHINSON, J. Encyclopedia of Anatomy and Physiology. London, 1849–1852.
2. HAMILTON, W. F. AND A. B. MORGAN. This Journal **99:** 526, 1932.
3. DOW, P. This Journal **127:** 793, 1939.
4. LEWIS, THOMAS. Exercises in Human Physiology. London, Macmillan Co., 1945.

ALVEOLAR GAS CHANGES DURING BREATH HOLDING[1]

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Although there are in the literature numerous studies of the maximum time that the breath can be held under various conditions (1), there is little information available regarding the changes in alveolar gas composition during breath holding. The composition of the alveolar air at the end of the involuntary apnoea (not the maximum voluntary apnoea) produced by two minutes of hyperventilation has been measured by Douglas and Haldane (2) with various concentrations of oxygen in the air inspired during the period of forced breathing. The same authors (3) as well as Hill and Flack (4, 5) have compared the alveolar compositions existing at the end of maximum breath holding when air and when pure oxygen were previously breathed. Ferris *et al.* (6) have measured the pCO_2 and pO_2 of the arterial blood at the 'breaking point' and recognize these factors as interrelated stimuli for termination of breath holding.

It is the purpose here to present data showing the alveolar gas exchange during periods of breath holding following the breathing of various inspired oxygen tensions and to consider the factors determining the maximum duration of breath holding.

METHODS

In all cases the breath was held at the end of a normal expiration in the sitting position. The same eight trained subjects[2] were used in all experiments. The breath was held as long as possible and the expiratory reserve was then vigorously blown through a large stopcock into a rubber tube two meters long and 2.5 centimeters in diameter. As soon as the expiration was complete the stopcock was closed to trap the sample of alveolar air in the tube. A small side arm just below the stopcock delivered the sample to the oxygen and carbon dioxide meters (7) where it was immediately analyzed.

Low oxygen tensions in the inspired air were obtained in a high altitude chamber by going in succession to simulated altitudes of 8, 12, 16 and 18 thousand feet. The subjects, who wore ear oximeters, remained at each altitude for about twenty minutes and delivered two samples which were analyzed at that altitude. Upon return to ground level the breath-holding test was repeated.

Oxygen tensions higher than normal were obtained by breathing 32 per cent and 45 per cent oxygen in nitrogen and 100 per cent oxygen from large spirometers at ground level for ten minutes before the breath was held. Each test was repeated twice or until reliable checks were obtained upon repeated trials.

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[2] We wish to acknowledge the fine cooperation of the members of Civilian Public Service Unit No. 115-R who served as subjects and technicians during these experiments.

RESULTS

At ground level, and particularly at the higher altitudes, there was no hesitation on the part of the subjects in deciding exactly when further apnoea was impossible. On the other hand, at the two highest oxygen concentrations it was often rather difficult to decide when the real breaking point had been reached. While holding the breath at these high oxygen tensions, the subjects experienced involuntary contractions of the respiratory muscles which became increasingly violent and rapid. At first they involved the diaphragm and later the intercostals and accessory breathing muscles. Simultaneously the subject experienced sensations of intense warmth with flushing of the skin and sweating, particularly of the forehead and of the palms of the hand. Even so, the first minute of apnoea on pure oxygen frequently seemed more uncomfortable than the last minute.

At the 18,000-foot altitude all subjects came very close to fainting. Some actually lost postural control momentarily with involuntary swaying of the body or head. Two men, however, were able to carry out the breath-holding maneuver successfully at 20,000 feet.

A summary of the average breath-holding times for eight subjects at 10 different tensions of inspired oxygen is given in table 1. Data are also given for ear oximeter readings at the breaking point, mean R.Q. during the period of apnoea and terminal values of alveolar pCO_2 and pO_2. In general the higher the O_2 tension, the higher the CO_2 at the breaking point and the longer the period of voluntary apnoea. The observed arterial saturations are all slightly lower than the values calculated from the alveolar gas tensions, presumably because the oximeter falls suddenly at the time of forcible expiration, as if from some circulatory disturbance, and we recorded only the lowest values.

The data relating to composition of the alveolar gas are graphically depicted in figure 1 by plotting them on a pCO_2-pO_2 diagram (8). The numbered circles indicate the average breaking points in the various experiments and form the basis for constructing the breaking point curve. This curve also adequately fits the breaking points determined from analysis of arterial blood by Ferris *et al.* (6). It is also similar in shape to the curve of Douglas and Haldane (2) showing the points at which involuntary apnoea following hyperventilation ceases, but is located higher on the diagram. The starting point for each test is indicated by the appropriate pCO_2-pO_2 value on the normal alveolar gas curve (constructed from our own data). The dotted lines leading from the starting points to the breaking points indicate the presumed time course of the alveolar gas changes during the breath-holding period. (The actual time course is known in only three subjects and will be discussed below.) Except for the lower tensions of inspired oxygen, the slopes of these lines become less with time, because the O_2 uptake from the lungs remains relatively constant while the CO_2 output di-

TABLE 1. SUMMARY OF ALVEOLAR GAS TENSIONS AND OTHER DATA IN BREATH HOLDING (B.H.) EXPERIMENTS

(1) Experiment no.	1	2	3	4	5	6[2]	7	8	9	10
(2) No. of subjects	8	8	8	8	8	8	8	8	6	7
(3) No. of expts.	12	16	16	16	16	16	22	15	13	14
(4) B-47 in mm. Hg	332	365	436	517	697	697	702	700	691	694
(5) Altitude in thousands of feet	18	16	12	8	0.5	0.5	0.5	0.5	0.5	0.5
(6) Inspired pO_2	69	76	91	108	146	146	147	227	317	694
(7) Inspired $O_2\%$	Air	Air	Air	Air	Air	Air	Air	32.4	45.9	100
(8) Alveolar pO_2 before breath hold	36	41	50	62	104	104	104	187[1]	277[1]	656
(9) Alveolar pCO_2 before breath hold	30	33	37	37	38	38	38	38	38	38
(10) Alveolar pN_2 before breath hold[5]	266	291	349	418	555	555	560	475	376	0
(11) Alveolar pO_2 after breath hold	28.0	31.1	36.1	44.2	62.7	45.9	52.1	72.5	157	628[3]
Std. dev. ±	1.0	2.9	1.4	3.0	9.9	6.7	9.0	16.0	34.2	3.9
(12) Alveolar pCO_2 after breath hold	38.6	42.3	45.6	46.9	50.1	48.3	50.2	57.5	62.6	66.3
Std. dev. ±	2.8	2.6	1.9	1.2	1.3	3.1	1.7	4.4	5.6	3.9
(13) Alveolar pN_2 after breath hold[5]	265	292	354	426	584	603	600	570	471	0
(14) Duration of B.H. in seconds	23	25	27	30	35	59	50	92	106	153
Std. dev. ±	6.4	3.7	6.4	8.7	4.4	18.3	17.1	28.9	38.9	53.5
(15) $HbO_2\%$ by oximeter	48	51	61	71	87	70				
(16) $HbO_2\%$ by calculation	55	58	67	77	90	78	86	92		
(17) Lung volume after B.H., liters	3.0	3.0	2.97	2.97	2.85	2.76	2.80	2.50	2.40	2.42[4]
(18) O_2 intake rate during B.H., cc/min.	73	84	109	143	264	220	230	289	300	
(19) Average R.Q. during breath hold	1.07	.93	.57	.48	.22	.10	.16	.08	.08	

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[1] Assumed 40 mm. Hg less than inspired pO_2 in row 6.

[2] Observations made immediately after hyperventilation caused by previous 'ascent' to altitude.

[3] Calculated as row 4 minus row 12. Measurements on the O_2 meter are very inaccurate at this level.

[4] Calculated by equation 6 assuming Q = 0.1.

[5] pN and p'N were calculated by subtracting the CO_2 and O_2 tensions from B-47 in row 4.

FIG. 1

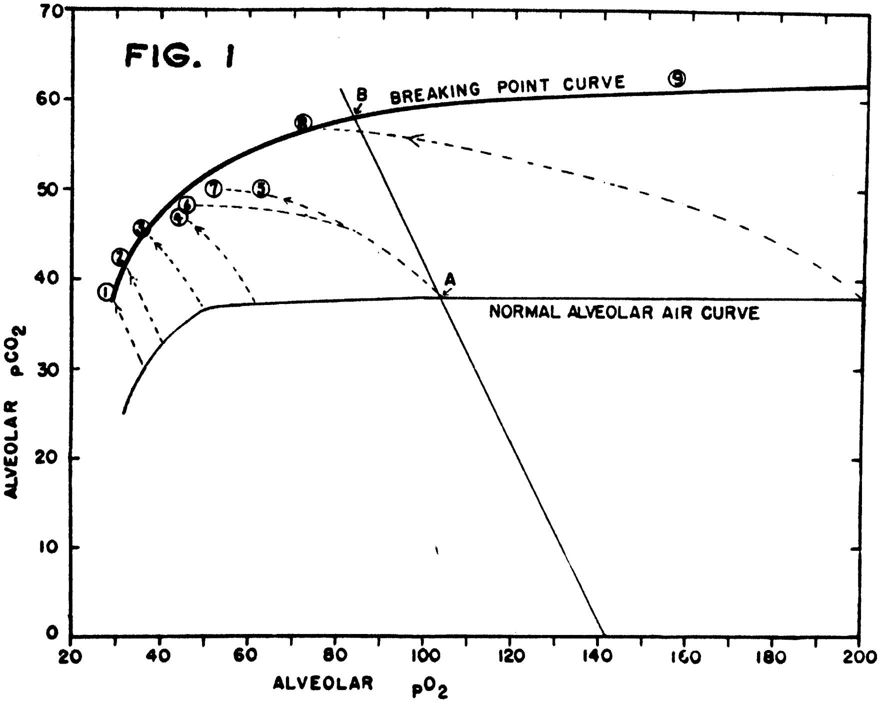

FIG. 2

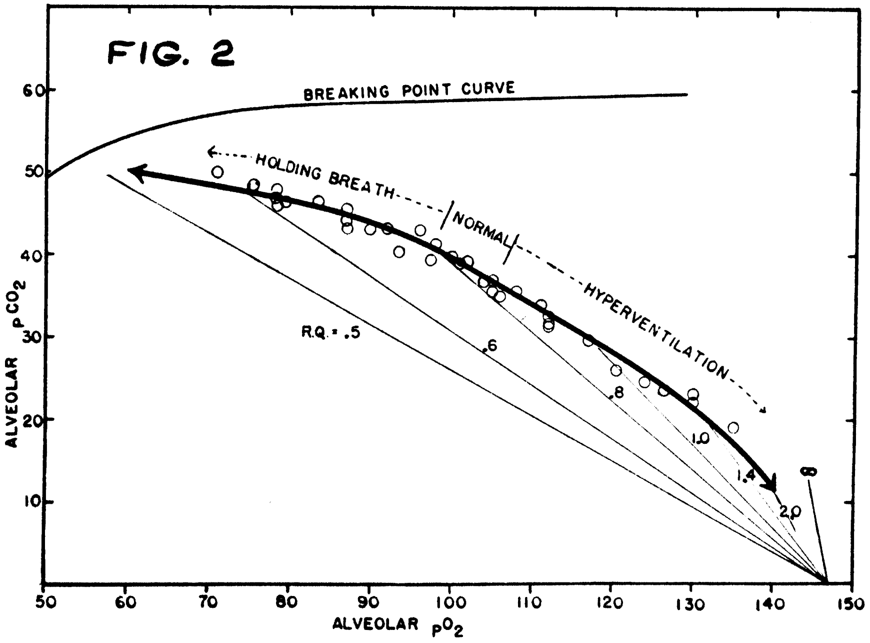

FIG. 1. THE PRESUMED COURSE OF CHANGE in alveolar gas composition during breath holding under various conditions.

FIG. 2. THE ALVEOLAR PATHWAY during breath holding and during hyperventilation.

minishes. This is explained by the fact that although an ample gradient for transfer of O_2 from lungs to blood exists throughout the breath-holding period, the gradient for transfer of CO_2 in the opposite direction becomes progressively less favorable owing to the ever increasing pCO_2 in the lungs.

At the lower inspired O_2 tensions, however, the slopes of these dotted lines are steeper in general and do not fall off much with time. At these O_2 tensions the alveolar diffusion gradient for oxygen is so much less than normal that the O_2 uptake from the lungs is lower and more nearly equal to the CO_2 transfer. Another manifestation of these processes is that the rate of change of lung volume during breath holding is greater the higher the pO_2 of the inspired air (9).

The dotted lines in figure 1 represent the presumed time course of the alveolar air composition while breathing air at different altitudes. The time course depends, however, not only upon the initial tension of oxygen but also on the percentage of oxygen. Point *A* in figure 1 represents the alveolar air at ground level, breathing air. The same tensions of oxygen and carbon dioxide can be obtained by breathing pure oxygen at an altitude of approximately 34,000 feet. In this case when the breath is held, the composition of the alveolar air must remain somewhere on the line AB, this being the diagonal for an R.Q. of 1.0 where $pCO_2 + pO_2 = 142$ (8). The carbon dioxide tension at point *B* is far higher than at point 7, breathing air. One of our subjects carried out this experiment and found that the pCO_2 at the breaking point was 48 mm breathing air at ground level but was 62.5 mm at 34,000 feet breathing pure oxygen. Likewise the pO_2 was higher and the period of voluntary apnoea longer in the latter case. This experiment confirms, therefore, the theoretical expectations. If the lungs contain pure O_2, a passage of O_2 into the blood causes a decrease in volume but no change in O_2 tension. In the presence of a large amount of N_2, however, a decrease in volume due to diffusion of O_2 results chiefly in increasing the pN_2 so that the O_2 tension becomes much less than when pure O_2 is breathed. This would tend to decrease the amount of CO_2 which can be tolerated and should shorten the breath-holding time. At the same initial oxygen tension, therefore, the greater the percentage of oxygen the less the decrease in pO_2 for the same O_2 consumption. As we have shown elsewhere (8) the R.Q. diagonals deviate less and less from the R.Q. = 1 line as the O_2 percentage increases until at 100 per cent O_2 they all coincide. The breath-holding experiment referred to is a manifestation of this phenomenon. The observation of Brown (10) that the breath can be held longer at 30,000 feet on pure O_2 than at ground level on air is also consistent with this, but the results of Rodbard (1) who found that breath-holding times on oxygen at 28,000 feet were less than at ground level on air cannot be explained on this basis.

The changes in the R.Q. and the sequence of the changes in alveolar composition during breath holding are shown in figure 2, which represents data on three subjects breathing air. Starting with an alveolar air represented by the region of the curve marked 'normal', the breath was held for various periods of time, after which the alveolar air was exhaled and analyzed. The alveolar point moves up the 'breath-holding curve' and gradually approaches the breaking point line.

In the other direction the line can be extended from the normal region to lower pCO_2 values by voluntary hyperventilation for different periods at a high rate. In this way the alveolar air changes so as to approach the point on the axis of abscissae from which the R.Q. lines radiate (8). This point represents the composition of the inspired air and can be reached only by an infinite ventilation rate. According to the chart, the R.Q. increases during hyperventilation. Thus the R.Q. is 1.4 where the hyperventilation curve intersects the 1.4 diagonal. When the breath is held the R.Q. becomes progressively less. In this case, however, the numerical magnitude of the R.Q. is not given by the diagonal R.Q. lines, because the original inspired air point can no longer apply when the glottis is closed, but rather by the slope (approximately) of the breath-holding curve. At the beginning of apnoea the slope of this curve is such that $\frac{\Delta pCO_2}{\Delta pO_2} = 0.5$. Since the pN_2 is fairly large in this case the total volume of the lung does not change much so that without serious error $\frac{\Delta pCO_2}{\Delta pO_2} = \text{R.Q.}$ The mean R.Q. during the whole period of apnoea is of course much less. Where the breath-holding curve crosses the R.Q. = 0.5 diagonal, the slope of the curve actually indicates an R.Q. of 0.15. The correct method of calculating the R.Q. from the slope of the breath-holding curve will be given below. (See equations 6 and 7.)

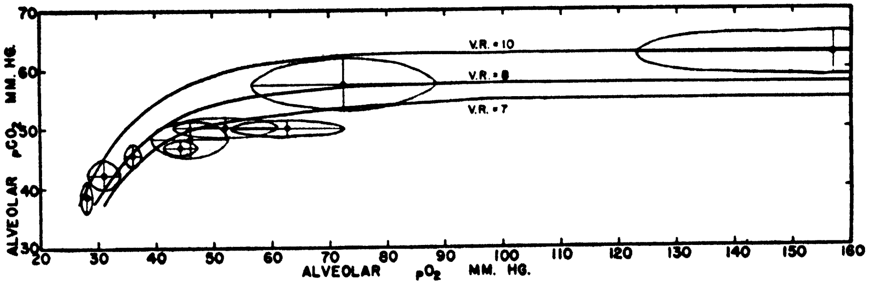

FIG. 3. THEORETICAL BREAKING POINT CURVES constructed as described in the text. The points are plotted from the experimental data in table 1. The standard deviation of each point is indicated by an ellipse.

DISCUSSION

For a more quantitative discussion of these results the following symbols will be used:

Let pO, pC and pN be alveolar tensions of O_2, CO_2 and N_2 before breath holding.

p′O, p′C and p′N = similar tensions at the breaking point

FC and F′C = the fraction of CO_2 in the lungs before breath holding and at the breaking point, respectively

V and V′ = lung volumes in cc. BTPS before and after breath holding.

x and y = cc. of O_2 lost and CO_2 added, respectively, during breath holding at BTPS.

B = ambient pressure in mm Hg
t = breath-holding time in minutes
X_o = cc. O_2 diffusing out of lung per min. at S.T.P.
Q = normal alveolar R.Q. preceding breath holding
Q′ = mean alveolar respiratory quotient during time *t*
W = an unknown virtual volume of body water
α = slope of the CO_2 dissociation curve for W. $\alpha = \frac{3500 \text{ cc. } CO_2 \text{ (S.T.P.)}}{\text{liter/atm.}}$

Calculation of alveolar oxygen consumption during breath holding

$$x = \frac{pOV - p'OV'}{B - 47} \tag{1}$$

$$X_0 = \frac{pOV - p'OV'}{(B - 47)t} \times \frac{273}{310} \times \frac{B - 47}{760}$$

$$= \frac{pOV - p'OV'}{863t} \tag{2}$$

The amount of nitrogen in the lung may be assumed to remain constant during breath holding,[3] so

$$p'NV' = pNV$$

and

$$V' = \frac{pN}{p'N} V \tag{3}$$

Substituting (3) in (2) gives

$$X_0 = \frac{\left(pO - p'O \frac{pN}{p'N}\right) V}{863t} \tag{4}$$

The average rates of oxygen consumption have been calculated for the breath-holding periods according to equation 4 by assuming an initial lung volume of 3000 cc. and the results are given in table 1. No value is given for experiment 10, because no nitrogen was present and equation 4 does not apply. Equation 2 could have been used in this case had the change in lung volume been known. It is apparent that the rate of oxygen consumption decreases from a normal value of 300 cc/min. during 106 seconds of breath holding at high oxygen tensions to 73 cc/min. for the 23-second period at 18,000 feet. A decrease in the intake of O_2 from the lungs indicates either that the metabolic rate is decreasing or that the O_2 reserves in the body (chiefly blood HbO_2) are being drawn upon. That the latter is more probable is indicated by the following argument.

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[3] We have calculated that in the experiments which involve the greatest change in pN_2 (columns 8 and 9 of table 1) the amount of nitrogen taken out of the lung by the blood would be less than 1 per cent of the total nitrogen present.

At 18,000 feet the breath was held for 23 seconds and during that time only 28 cc. of O_2 were removed from the lungs instead of the normal value of about 120 cc. At the same time, however, the arterial saturation fell from a probable value of 70 per cent to an observed value (oximeter) of 48 per cent. If the average fall in all the blood (including venous) were only half as great, at least 10 per cent of the oxygen capacity of the blood or about 100 cc. could have been consumed in the tissues. It may be supposed, therefore, that the O_2 actually used by the tissues does not decrease during the period of breath holding even at anoxic altitudes.

Calculation of the mean alveolar R.Q. during breath holding

$$y = \frac{p'CV' - pCV}{B - 47} \tag{5}$$

$$Q' = \frac{y}{x} = \frac{p'CV' - pCV}{pOV - p'OV'} \tag{6}$$

Substituting (3) in (6)

$$Q' = \frac{p'C\,\dfrac{pN}{p'N} - pC}{pO - p'O\,\dfrac{pN}{p'N}} \tag{7}$$

Equation 7 has been used to calculate the mean R.Q. during the breath-holding period for all experiments where nitrogen was present. The results are recorded in table 1. At 18,000 and at 16,000 feet the average R.Q. for the period of apnoea is 1.07 and 0.93, respectively, but at ground level on air the value is 0.1 to 0.22 and when extra O_2 is added, values as low at 0.05 result. When the R.Q. is less than unity, there is a decrease in lung volume during the breath-holding period. The lung volume at the end of the breath-holding period may be computed by equation 3. Results of this calculation, assuming an initial lung volume of 3.0 liters, are recorded in table 1. When the breath is held on pure O_2, equations 3 and 7 do not apply, but a relationship between lung volume change and R.Q. is given by equation 6. The final lung volume in experiment 10 has been estimated by the use of this equation, assuming an R.Q. of 0.1 and an initial lung volume of 3.0 liters.

The lung volume is not significantly lowered during breath holding on air at high altitudes, because the R.Q. is about unity, but at ground level and at high oxygen tensions the volume decreases from an initial value of 3.0 liters to a final value of 2.4 liters. Since the breath was held at the end of a normal expiration, this means that the final lung volume is 600 cc. less than the relaxation volume and that some expiratory effort is required to avoid the development of negative pressure within the lungs (11). Breath holding on pure oxygen at high altitudes involves an even greater decrease in volume. We have not investigated this matter thoroughly, but one subject who held his breath on oxygen at 34,000 feet was unable, at the breaking point, to expire enough gas to give a sample for analysis. It is probable that under such conditions, a decrease to the residual volume might be the decisive factor terminating breath holding.

The high R.Q. values observed during breath holding at anoxic altitudes are the result of the decreased oxygen consumption that occurs under these conditions as described above. The low R.Q. values obtained at ground level where the oxygen consumption remained normal are due to retention of CO_2 in the blood and other tissues, the rising pCO_2 in the lungs creating a less favorable gradient for CO_2 transfer. The amount of CO_2 retained depends on the relative volumes involved of lung air and tissue water, on the slope of the CO_2 dissociation curve of the tissue water and on the length of time available for equilibration.

The total CO_2 (S.T.P.) formed during a period of breath holding is given by

$$(F'CV' - FCV)\left(\frac{B - 47}{760} \times \frac{273}{310}\right) + (F'C - FC)\alpha W \tag{8}$$

If the alveolar O_2 consumption remains the same as before breath holding.

$$\frac{(F'CV' - FCV)\,\frac{(B - 47)}{(863)}}{(F'CV' - FCV)\,\frac{(B - 47)}{(863)} + (F'C - FC)\alpha W} = \frac{Q'}{Q} \tag{9}$$

Multiplying by $\frac{B - 47}{B - 47}$ to convert 'F' to 'p' gives

$$\frac{(p'CV' - pCV)\,\frac{(B - 47)}{(863)}}{(p'CV' - pCV)\,\frac{(B - 47)}{(863)} + (p'C - pC)\alpha W} = \frac{Q'}{Q} \tag{10}$$

If values from table 1 for experiment 5, in which the breath was held on air at ground level for 35 seconds, are substituted in this equation, *W* is found to be 1.57 liters. For experiment 9, in which the breath was held 106 seconds on a higher oxygen mixture, *W* comes out to be 3.08 liters. The value of 3500 for α is the slope of the CO_2 dissociation curve for blood at 45 mm Hg from Field *et al.* (12). Some justification for using this value for the tissues is found in a paper by Adolph *et al.* (13), who found that for a given rise in CO_2 tension, 3–18 per cent of the CO_2 was absorbed by the blood and 82–97 per cent or about nine times as much, on the average, by the tissues. Since the total volume of body water is about nine times the blood volume, the slope of the CO_2 dissociation curves for blood and tissues must be about equal. If α is taken as only half that of blood, as indicated by the experiments of Shaw (14), the values of *W* will be doubled, but at any rate these calculations indicate that the CO_2 formed during breath holding equilibrates virtually with only a small fraction of the total 40 or 45 liters of body water. The blood volume alone is ample to account for it.

Actually, of course, the total blood volume is not equilibrated nor is any other tissue. The blood leaving the lungs has a progressively higher pCO_2 as breath holding continues, and the tissues with the best circulation will be most nearly equilibrated. The more actively metabolizing tissues such as brain and kidney will not only produce more of the CO_2 formed during breath holding but will also retain a larger amount, because of their more rapid circulation. Some tissues may be so slowly circulated that during the shorter periods of breath holding they are not affected at all. For a tissue to retain CO_2 during the breath-holding period the duration must be at least as long as the circulation time from the lungs to that tissue. The steady decrease with time in the value $\frac{\Delta pC}{\Delta pO}$ that occurs when the oxygen uptake from the lungs remains constant during breath holding is due, therefore, to retention of CO_2 in progressively greater fractions of the body water. Presumably if the breath were held long enough to stop all O_2 consumption and CO_2 production, and if the ciruclation continued long enough, the pCO_2 in the lungs would slowly fall, as the poorly circulated areas of low metabolic rate gradually took up from the lungs their share of the CO_2 formed during the apnoea. A slight fall of alveolar pCO_2 has in fact been observed in obstructive asphyxia by Thompson and Birnbaum (15) and by Grodins *et al.* (16), but in similar experiments on dogs in this laboratory (17) the heart apparently stopped before the fall could be recorded.

The theoretical breaking point. Gray (18) has shown how various stimuli and inhibitions to breathing and their inter-relationships may be expressed in a quantitive fashion. The success with which Gray applied his approach to various respiratory problems suggests the application of his methods to the phenomenon of breath holding.

Breath holding is an example of the voluntary inhibition of an ordinarily involuntary process. It seems reasonable to assume that if one could express inhibition in quantitative units, maximum voluntary inhibition would be a constant value independent of the nature of the opposing stimuli. The breaking point in breath holding might then be considered as the point at which the stimulus to breathe becomes equal to the maximum voluntary inhibition.

The principal stimuli acting during breath holding are probably the pCO_2, the H ion concentration, and the pO_2 of the arterial blood. Gray has shown that the partial effects of each of these stimuli may be computed independently and that the net total stimulus is the algebraic sum of all three. The *chemical ventilation equation* describing this relationship is:

$$\text{V.R.} = 0.22\,\text{H} + 0.262\,\text{pCO}_2 - 16 + \frac{105}{10^{0.038\,\text{pO}_2}}$$

where *V.R.* is the ventilation ratio or the ratio of the observed to the normal alveolar ventilation. If the bicarbonate capacity is normal, *H* and *pCO_2* are so related that the above equation is simplified to:

$$\text{V.R.} = 0.4\,\text{pCO}_2 - 15 + \frac{105}{10^{0.038\,\text{pO}_2}}$$

This equation applies exactly to steady state conditions only. Since no equation of this sort has yet been derived to describe the particular unsteady state that exists during breath holding, the above equation has been used to estimate the stimulus strength existing at the breaking point for each set of data in table 1. The results of these calculations are shown in table 2. The different values obtained for the total stimulus at the breaking point are hardly constant as would be demanded by an hypothesis of a constant maximum voluntary inhibition. This discrepancy may be due to the inaccuracy previously pointed out of applying a steady state equation to an unsteady state, to a less close approach to equilibrium between alveolar air and arterial blood during breath holding than during normal breathing, (although the close correspondence between our breath-holding curve based on alveolar air values and a similar curve based on arterial blood values (6) would seem to minimize this) and possibly to the action of stimuli such as reflexes from the lungs and formation of lactic acid which are not taken into account in the equation. The calculated partial stimulus effects of pCO_2 and of pO_2 in each case are probably relatively correct and indicate that the stimulus at the breaking point in the various experiments of table 1 may be *a*) completely due to high pCO_2, *b*) almost completely due to low pO_2 or *c*) due to various combinations of hypercapnic and hypoxic stimuli. The mean value for the total stimulus at the breaking point (V.R. = 8) has been used as a basis for constructing a theoretical curve for breath holding. By the method indicated in table 3 various pCO_2, pO_2 combinations that should produce a total V.R. = 8 have been calculated. The theoretical breaking point curve for breath holding

TABLE 2. THE THEORETICAL VENTILATION STIMULUS EXISTING AT THE BREAKING POINT OF BREATH HOLDING

pCO_2	pO_2	$V.R._{H,pCO_2}$	$V.R._{pO_2}$	$V.R._{Total}$
38.6	28.0	0.4	9.1	9.5
42.3	31.1	1.9	6.9	8.8
45.6	36.1	3.3	4.3	7.8
46.9	44.2	3.8	2.2	6.0
50.1	62.7	5.0	0.4	5.4
48.3	45.9	4.3	1.9	6.2
50.2	52.1	5.1	1.1	6.2
57.5	72.5	8.0	0.2	8.2
62.6	157.	10.0	0	10.0
66.3	527.	11.5	0	11.5
Mean................				8.0

Explanation of column headings:

pCO_2 and pO_2 refer to the alveolar gas tensions in mm. Hg measured at the breaking point.

$V.R._{HpCO_2} = 0.4pCO_2 - 15$, and is the partial ventilation stimulus due to the H ion, pCO_2 complex.

$V.R._{pO_2} = \frac{105}{10^{0.038pO_2}}$ and is the partial ventilation stimulus due to pO_2.

$V.R._{Total} = V.R._{H,pCO_2} + V.R._{pO_2}$ and is the total stimulus to breathe at the breaking point. According to the proposed hypothesis, it is also equal to the maximum voluntary inhibition.

has been plotted from these calculated pCO_2, pO_2 combinations on figure 3 (curve labeled V.R. = 8) along with the original experimentally determined points. Two other similarly constructed curves assuming constant stimuli of V.R. = 7 and V.R. = 10, respectively, are shown on the same graph for comparison. These curves are of approximately the proper shape, and except at the higher CO_2 tensions, the fit of the curve for V.R. = 8 is hardly worse than the scatter of the original data, which is indicated by the ellipses of standard deviation surrounding each point.

Although the hypothesis that the breaking point of breath holding occurs when the total stimulus to breathe becomes equal in value to a constant maximum voluntary inhibition is not proven by the above treatment, the results are gratifying enough to suggest further work. For example, the curve for the breaking point should be displaced downwards and to the right in persons acclimatized to altitude because, as Gray has pointed out, the ventilation of such individuals has increased sensitivity to CO_2. Exercise, either active or passive, should also similarly displace the curve because a strong reflex stimulus to breathe would be added to the H, pCO_2, pO_2 combination. The maximum voluntary inhibition estimated above and expressed as V.R. = 8 is close to the maximum ventilation rate attained during active muscular exercise. If a person exercised at a rate such that his ventilation equalled the maximum voluntary inhibition, then he should not be able to stop breathing at all. His breath-holding time would be zero.

TABLE 3. CALCULATED pCO_2, pO_2 VALUES THAT WILL GIVE A TOTAL VENTILATION RATIO EQUAL TO 8

$V.R._{H,pCO_2}$	$V.R._{pO_2}$	pCO_2	pO_2
8	0	57.5	100
7	1	55.0	53.2
6	2	52.5	45.2
5	3	50.0	40.5
4	4	47.5	37.4
3	5	45.0	34.7
2	6	42.5	32.6
1	7	40.0	31.0
0	8	37.5	29.3

Explanation of column headings:

$V.R._{H,pCO_2}$ refers to the partial stimulus effect assumed due to the H ion, pCO_2 complex.

$V.R._{pO_2} = 8 - V.R._{H,pCO_2}$

pCO_2 was calculated from the equation, $V.R._{H,pCO_2} = 0.4\ pCO_2 - 15$

pO_2 was calculated from the equation $V.R._{pO_2} = \frac{105}{10^{0.038 pO_2}}$

SUMMARY

1. Breath holding was investigated in eight subjects at inspired oxygen tensions varying from 69 to 694 mm Hg. The time, HbO_2 per cent, CO_2 and O_2 alveolar tensions were recorded at the breaking point.

2. At high O_2 tensions the breath-holding time was long, the R.Q. was 0.05 to 0.1 due to retention of CO_2 and the rate of O_2 consumption during apnoea was normal.

3. At low O_2 tensions (18,000 feet) the breath-holding time was short, the rate of O_2 consumption only about one-fourth the normal, so that the R.Q. was about 1.0.

4. When R.Q. is less than 1.0 the lung volume tends to decrease. If N_2 is present this results chiefly in increasing pN_2 so that pO_2 decreases rapidly. When N_2 is absent the decrease in volume chiefly prevents the pO_2 from falling, so that the pCO_2 is much higher at the breaking point for the same initial alveolar pO_2.

5. Theoretical treatment of the data indicates that the breaking point of breath holding occurs when the sum of the separate partial stimuli from low pO_2 and from high pCO_2 gives a total stimulus sufficient to produce an alveolar ventilation about eight times the normal.

REFERENCES

1. Rodbard, S. This Journal **150:** 142, 1947.
2. Douglas, C. G. and J. S. Haldane. J. Physiol. **38:** 401, 1909a.
3. Douglas, C. G. and J. S. Haldane. J. Physiol. **38:** 420, 1909b.
4. Hill, L. and M. Flack. J. Physiol. **37:** 77, 1908.
5. Hill, L. and M. Flack. J. Physiol. **40:** 347, 1910.
6. Ferris, E. B., G. L. Engel, C. D. Stevens and J. Webb. J. Clin. Invest. **25:** 734, 1946.
7. Rahn, H., J. Mohney, A. B. Otis and W. O. Fenn. J. Aviation Med. **17:** 173, 1946.
8. Fenn, W. O., H. Rahn and A. B. Otis. This Journal **146:** 637, 1946.
9. Stevens, C. D., E. B. Ferris, J. Webb, G. L. Engel and M. Logan. J. Clin. Invest. **25:** 723, 1946.
10. Brown, E. B. U. S. Naval Air Base Research Report, Pensacola, Florida, Oct. 5, 1944.
11. Rahn, H., A. B. Otis, L. E. Chadwick and W. O. Fenn. This Journal **146:** 161, 1946.
12. Field, H., A. V. Bock, E. F. Gilden and F. L. Lathrop. J. Clin. Invest. **1:** 65, 1924.
13. Adolph, E. F., F. D. Nance and M. S. Shiling. This Journal **87:** 532, 1929.
14. Shaw, L. A. This Journal **79:** 91, 1926.
15. Thompson, S. A. and G. L. Birnbaum. J. Thorac. Surg. **12:** 607, 1943.
16. Grodins, F. S., A. Lein and H. Adler. This Journal **147:** 433, 1946.
17. Herber, Florence. Personal communication, 1946.
18. Gray, J. S. Science **103:** 739, 1946.

METABOLIC CHANGES OF BLOOD AND TISSUE GASES DURING ASPHYXIA[1]

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Recently several investigators (1–4) have questioned the alleged benefits of 5 to 7 per cent CO_2 in oxygen as a resuscitating agent for cases of obstructive asphyxia. The present study was undertaken, first, in order to confirm evidence concerning the acid-base balance of the blood during obstructive asphyxia, and thereby to aid in the evaluation of the CO_2-O_2 mixture as a resuscitating agent in such cases. Secondly, we have expanded that evidence to include a simultaneous study of blood lactic acid levels, (a measure of the degree of metabolic acidosis during asphyxia), and other pertinent variables to be indicated. Thirdly, we have made a preliminary study of the metabolic activity of muscle tissue during obstructive asphyxia. Efforts have been made to correlate it with simultaneous acid-base variations in the blood.

METHODS

The study of 10 healthy dogs, anesthetized by intraperitoneal injections of Dial[2] to surgical anesthesia, is reported here. The trachea was cannulated, and each dog was asphyxiated by rebreathing from a small spirometer a quantity of room air approximately equal to four times its tidal volume. Respiratory excursions throughout the asphyxiating procedure were recorded on a moving drum by a writer attached to the spirometer bell. A 5 cc. alveolar air sample was withdrawn from the rebreathing tube into an airtight syringe every one or two minutes during the asphyxiating procedure. Just before each sample was drawn, the chest was forcibly compressed, and held in this position during the sampling. The syringes were inverted in tubes of mercury, and so sealed until analyzed for CO_2 content within 24 hours by the Fry (5) gas analyzer.

Every one or two minutes during the asphyxiating procedure a 5–6 cc. sample of arterial blood was withdrawn from the carotid artery through a blunted needle into a syringe, the dead space of which had been filled with 6.5 percent NaF. The exact amount of fluoride in each syringe was determined, and this dilution factor considered when calculating blood concentration from the analytical figures. Approximately 6 mgm. of fluoride per cc. of blood was found to be sufficient to prevent clotting and glycolysis for 48 hours, if the blood was stored

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[1] Work done under contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of Rochester, and under contract with the Air Materiel Command, Wright Field.

[2] Each cc. containing 0.1 gram diallylbarbituric acid, 0.4 gram urethane, 0.4 gram monoethylurea, and q.s. distilled water.

in ice water. The withdrawal and storage of the blood was done without exposing it to air. The CO_2 and O_2 contents of 0.5 cc. arterial samples were measured by the Van Slyke and Neill (6) manometric technique of blood gas analysis. Arterial pH was determined at room temperature by the Beckman pH meter in a capillary glass electrode, without exposure to air. The hematocrit ratios were determined on duplicate samples of arterial blood, centrifuged exactly 20 minutes at 4000 r.p.m. The arterial blood of *dogs 4* to *10* was analyzed for lactic acid, according to the Barker and Summerson (7) technique, and the color concentration read by the Klett-Summerson colorimeter, using a 460 mu filter.

One-half hour before the beginning of asphyxia, the tibialis anterior and peroneus longus muscles in each hind leg of *dogs 8, 9*, and *10* were dissected free, leaving blood and nerve supply intact. The skin flaps were closed to permit the reestablishment of normal gas content of the muscles. Two samples, each approximately 1 gram in size, were cut from each isolated muscle and the two corresponding samples from the other leg at death. The transfer of tissues to stoppered containers of CO_2-free FeF_3 was made as quickly as possible (15 to 20 seconds from the time the skin flap was opened). Muscle CO_2 content was determined by the method of Danielson and Hastings (8), a modification of the Van Slyke manometric technique for blood gas analysis.

Electrocardiographic records (lead I only) were taken for 20 seconds out of every one or two minutes, i.e., at exactly the same time as blood and alveolar air were sampled by assistants in the experiment.

Muscles samples were analyzed within 18 hours after the experiment, and blood samples within 24 hours. The proteins and sugars were precipitated for the lactic acid determination within 24 hours, though completion of these analyses was often delayed up to 72 hours.

No attempt was made to resuscitate the animals, but blood samples were withdrawn as long as the arterial pressure would permit, and alveolar air samples were taken over the same length of time.

RESULTS

The mean values of each variable measured (with the exception of muscle CO_2), as well as their standard deviations, are presented in table 1. There was a wide variation from animal to animal in survival time; the mean figures for each variable therefore have progressively less reliability after the seven-minute interval. The same mean values are plotted in figure 1 as percentage change from the control values against time after the rebreathing began.

The mean curve of pulmonary ventilation reached its peak 4 minutes after rebreathing began. Respiration began to fail from 3½ to 6 minutes, and completely ceased from 4½ to 7½ minutes after asphyxia began. Alveolar CO_2 continued to rise throughout the experiments, from a control mean of 6.48 percent to 14.10 percent at death. Every animal showed a progressive rise in alveolar CO_2 content.

Though the arterial pressure was not regularly recorded, in all cases it dropped so low that no more blood could be withdrawn. The average critical period between cessation of respiration and failure of blood pressure to the point where no more blood could be drawn was 2.7 minutes.

A mean rise in whole blood CO_2 content from 41.5 to 57.1, or a change of 15.6 vol. percent, occurred during the first 8 minutes, followed by a fall to 52.1, at the end of 10 minutes. The decreasing CO_2 content was shown by 7 of the 10 dogs reported here. Mean blood oxygen fell from a control level of 18.03 vol. percent to 0.33 vol. percent, 69.4 percent of the oxygen being lost during the first four minutes of asphyxiation, and 89.4 percent within the first six minutes. A temperature correction of −0.01 pH unit per 1°C. rise was applied to the pH values measured at room temperature (9). The results indicate a steady fall in arterial pH from a control mean of 7.26 to a terminal value of 7.00.

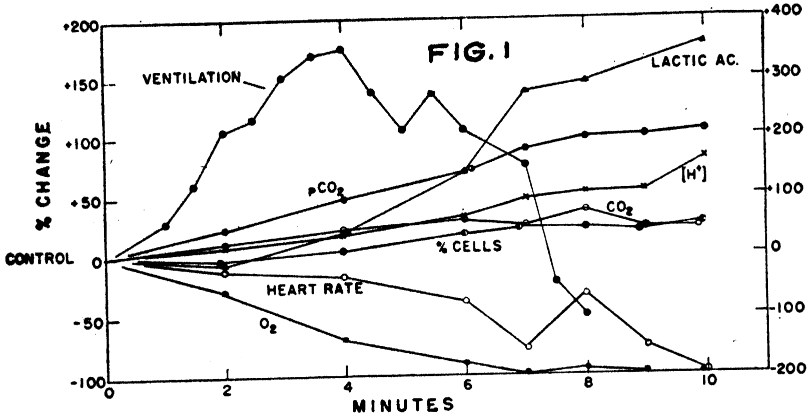
TABLE 1. Mean experimental data on asphyxiated dogs

Each figure is an average of data from 8 to 10 dogs, unless otherwise stated. The standard deviations are given below each figure.

	NORMAL	2 MIN.	4 MIN.	6 MIN.	7 MIN.	8 MIN.	9 MIN.	10 MIN.
Respiration analyses								
% CO_2 in alveolar air	6.48	8.44	10.07	11.83	13.80[1]	13.67[1]	14.17[2]	14.10[2]
σ	0.74	0.70	1.05	1.39	0.35	1.80	1.62	1.84
Alveolar pCO_2 in mm. Hg	48.0	59.3	70.7	83.5	90.3[2]	95.1[1]	97.8[1]	98.5[2]
σ	4.51	4.35	7.22	8.95	13.14	12.86	12.24	24.36
Pulmonary ventilation in cc/min.	1153	3618	5236	3596[2]	2892[2]			
σ	280	198	2432	2462	634			
Arterial blood analyses: whole blood as drawn								
Vol % CO_2	41.51	46.35	50.78	53.59	51.80[2]	57.07[2]	51.0[2]	52.1[2]
σ	5.55	4.97	5.43	6.03	2.40	2.99	8.63	4.36
Vol % O_2	18.03	13.04	5.54	1.92	0.37[2]	0.99[2]	0.44[2]	0.33[2]
σ	2.70	1.73	3.35	1.82	0.31	0.44	0.14	0.18
pH 37°	7.26	7.22	7.19	7.13	7.09[2]	7.07[2]	7.07[2]	7.00[2]
σ	0.04	0.06	0.05	0.05	0.06	0.09	0.07	0.05
Hematocrit	39.4	37.7	41.2	43.9	49.2[2]	48.3[2]	47.6[2]	50.5[3]
σ	6.6	8.1	4.6	7.2	2.2	2.2	6.1	3.8
Lactic acid content in mEq/L	1.22	1.15	1.49	2.08[1]	2.92[2]	3.10[2]		3.40[2]
σ	0.58	0.45	0.47	0.85	0.80	1.28		1.85
Heart rate/min.	140	126	117	88	31[2]	94[2]	34[2]	
σ	30.3	35.5	30.9	41.1	17.0	45.3	10.3	

[1] 6 or 7 animals averaged. [2] Less than 6 animals averaged. [3] 2 animals averaged.

Control hematocrit values spread from 30.8 to 52.1 percent. As asphyxia progressed, however, the percentage in each dog rose, resulting in a mean increase of 28.2 percent during 10 minutes of asphyxia.



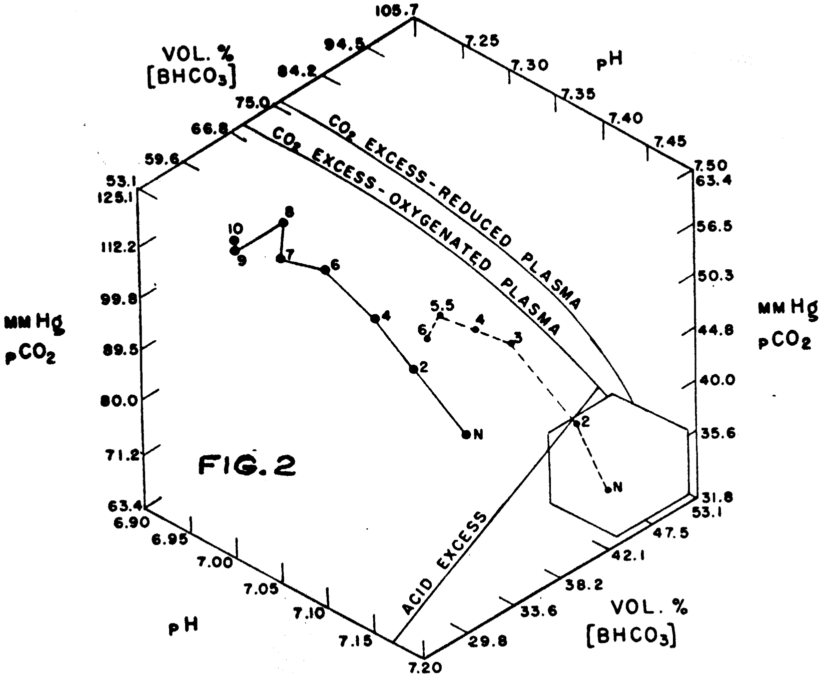


Fig. 1. Summary of variables observed during asphyxia. The mean values of each variable are expressed as per cent change from the control. On the right is the scale for pulmonary ventilation. All other variables are plotted using the left hand scale.

Ventilation = cc. pulmonary ventilation/minute; pCO_2 = alveolar tension in mm.Hg; Lactic ac. = mg. lactic acid/100 cc. whole blood; O_2 = cc. O_2/100 cc. whole blood; CO_2 = cc. CO_2/100 cc. whole blood; (H^+) = hydrogen ion concentration of plasma at 37° C.; % cells = hematocrit (4000 r.p.m. for 20 minutes); Heart rate = recorded by electrocardiogram.

Fig. 2. Acid-base balance of the blood during asphyxia. The small interior hexagon represents the range of normal variation of dogs, as determined by Grodins, Lein, and Adler, this Journal **147**: 433, 1946 (see text). The solid line represents the mean acid-base displacement of our dogs during asphyxia. (The calculations of the $(BHCO_3)$ value of the last (10 min.) point by the Van Slyke and Sendroy nomogram make use of only two hematocrit values, separated by 8.9 per cent. Calculations using individual hematocrit values of 46.0 and 54.9 per cent give values of 52.6 and 54.4 $(BHCO_3)$ respectively). The dashed line indicates the mean acid-base path of Grodins' asphyxiated dogs, reproduced from available data and graphs.

Lactic acid content of the arterial blood rose from a mean control level of 11 mgm. percent to 30.6 mgm. percent after 10 minutes of asphyxiation. The rise in lactic acid content did not become marked, however, until after about 6 minutes of asphyxiation, following which it rose very rapidly. This rise accompanied the fall of arterial oxygen below five volumes percent. This same relationship was pointed out by Scholander (10, 11) and Grinnell, Irving, and Scholander (12) on arrested breathing experiments with several species of animals.

The control heart rate, as recorded by the string galvanometer, was exceedingly variable from animal to animal, though the heart rate of each animal gradually decreased as asphyxia progressed. Cardiac change occurred, after respiration had ceased, usually in the form of auricular fibrillation and complete heart block. A supraventricular tachycardia that cannot further be defined is the cause of the increase in mean heart rate at the eight-minute interval.

TABLE 2. DOG MUSCLE CO_2 CONTENT—CONTROL AND DEATH FROM ASPHYXIA

EXPERIMENT NUMBER	MUSCLE ANALYZED	TOTAL CO_2 IN VOL. PERCENT		COMBINED CO_2 IN VOL. PERCENT		
		Normal	Death	Normal	Death	Av. Δ CO_2
8	Tibialis anterior	36.5	31.6	33.9	26.4	
		29.0	25.0	26.4	19.8	−7.0
	Peroneus longus	32.8	37.0	30.2	31.8	
		35.6	29.9	33.0	24.7	−3.3
9	Tibialis anterior	36.7	37.1	33.6	30.8	
		34.2	37.2	3[illegible].1	30.9	−1.5
	Peroneus longus	33.4	38.6	30.3	32.3	
		38.0	29.1	34.9	22.8	−5.0
10	Tibialis anterior	—	33.1	—	27.4	
		33.8	33.5	31.0	27.8	−3.2
	Peroneus longus	36.2	35.9	33.4	30.2	
		37.9	38.6	35.1	32.9	−2.7
Mean		34.9	33.9	32.1	28.2	
Average Δ CO_2						−3.8

Complete muscle data are found in table 2. The mean difference between samples of the same muscle is 3.04 vol. percent, and the σ of the difference is 2.12. Total CO_2 was analyzed directly by the Danielson and Hastings technique. The combined CO_2 content was obtained by subtracting from the total CO_2 content the amount of CO_2 dissolved at the pCO_2 to which the muscle was subjected. This assumes a CO_2 absorption coefficient in muscle of 0.41, after Irving, Foster, and Ferguson (13). Muscle pCO_2 was estimated as arterial (alveolar) pCO_2 plus 6 mm. Hg (A-V difference), on the doubtful assumptions that the A-V pCO_2 difference is a constant in spite of different circulation rates, and that muscle pCO_2 is the same as venous pCO_2.

DISCUSSION

Our experimental data concerning the acid-base balance of the blood, as expressed by the Henderson-Hasselbalch equation, $\mathrm{pH} = \mathrm{pK}_1 + \frac{\log\ (\mathrm{BHCO_3})}{\log\ (\mathrm{H_2CO_3})}$, have been plotted as a solid line on the logarithmic triaxial graph (fig. 2) introduced by Shock and Hastings (14). The inner hexagon represents the normal acid-base range of dog blood determined by Grodins *et al.* The pathways of CO_2 excess represent averages of *in vitro* CO_2 absorption curves of oxygenated and reduced true plasma (Shock and Hastings, 15; taken from the data of Henderson, 16). These pathways, as well as the fixed acid excess path, are in accordance with *in vivo* experimental observations of Shock and Hastings (15).

Our data are plotted from alveolar pCO_2, measured directly, and plasma $(BHCO_3)$, calculated from whole blood $(BHCO_3)$ by the Van Slyke and Sendroy (17) nomogram. This nomogram makes use of mean hematocrit values for each time interval. The mean figure for the last (10 min.) point is an average of only two hematocrit values, separated by 8.9 percent. Nomogram calculations making use of the individual hematocrits, 46.0 and 54.9 percent, give values of 52.6 and 54.4 vol. percent $(BHCO_3)$, respectively.

It will be noted that the pH values so determined agree within 0.05 pH unit with experimental pH values corrected to 37°C., (with the exception of the mean figure for the nine-minute interval). A depression of the respiratory center, due to anesthesia alone, must account for the relatively acid pH and high pCO_2 of the 'normal' sample.

The acid-base displacement curve of our dogs agrees very closely with that of Grodins *et al.*, who asphyxiated dogs by completely obstructing the trachea, and whose results are shown approximately as a dashed line on figure 2.

Our data, with those of Grodins *et al.*, can best be described as representing an initial respiratory acidosis, associated with decreasing pH, increasing $(BHCO_3)$, and increasing pCO_2. Superimposed on the respiratory acidosis toward the end of asphyxia is a slight uncompensated metabolic acidosis, characterized by a decreasing pH, decreasing $(BHCO_3)$, and increasing pCO_2. The source of metabolic acidosis in these experiments appears to be an accumulation of lactic acid and other fixed acids from the anaerobic glycolysis taking place in the absence of oxygen, and displacing CO_2 from the blood. The accumulation of lactic acid in the blood is shown by the direct measurements of lactic acid content indicated in table 1.

Approximately 2 percent of the 28.2 percent rise in hematocrit can be accounted for by the shifting of water from plasma to cells, accompanying the acid-base changes of the blood during the experiment (16). The remaining 26.2 percent is explained by two possible mechanisms, neither of which was actually observed in these experiments, but both of which are known to occur in asphyxia: 1) discharge of red blood cells from the spleen into the circulation in response to lowered oxygen tension and 2) leakage of fluid and whole plasma into tissues, and a consequent hemoconcentration associated with shock. Thompson and

Birnbaum (2) have found regular evidence of shock in dogs subjected to obstructive asphyxia. Grodins *et al.* report an increase in hemoglobin content of 24.6 percent during obstructive asphyxia in dogs.

Inspection of table 3 reveals that the CO_2 content of the muscles is no higher at the end of asphyxia than at the beginning, as would be the case if these muscles stored the CO_2 displaced from the blood; rather, the mean death sample CO_2 content is 3.8 vol. percent lower than the control, despite increasing CO_2 tensions in the muscles. Each figure for muscle CO_2 content in table 3 is an average of four CO_2 analyses: two of tibialis anterior and two of peroneus longus. By comparison of our muscle CO_2 data with the CO_2 dissociation curve and muscle buffering power data found for dog muscle by Irving, Foster, and Ferguson, we have estimated the amount of fixed acid necessary to account for the decrease in muscle CO_2 content, despite increased muscle pCO_2, as asphyxia progressed. For this purpose it has been assumed, as previously, that muscle pCO_2 equals arterial (alveolar) pCO_2 plus 6 mm. Hg. Muscle pH is calculated from the Henderson-Hasselbalch equation, the pK_1 value being 6.1. Recent evidence of Conway and Fearon (18) for carbamino-bound CO_2 in muscle would make these pH values considerably lower, though the relative changes would be the same.

TABLE 3. SUMMARY OF DOG MUSCLE EXPERIMENTS

EXPERIMENT NUMBER	COMBINED CO_2 IN MUSCLE	ARTERIAL pCO_2 PLUS 6 mm. Hg	MUSCLE pH	ESTIMATED LACTIC ACID CONTENT OF MUSCLE AT DEATH	LACTIC ACID CONTENT OF ARTERIAL BLOOD AT DEATH
	Vol %	*mm. Hg*		*mEq/L.*	*mEq/L.*
8 Control	30.9	48.5	7.17		
Death	25.7	95.9	6.79	9.65	3.40
9 Control	32.5	56.6	7.12		
Death	29.2	116.0	6.80	8.68	5.24
10 Control	33.2	51.0	7.18		
Death	29.6	105.4	6.85	9.05	2.71

The estimated muscle lactic acid content at death (table 3, column 5) varies from 8.68 to 9.65 mEq./liter. Simultaneous lactic acid contents of the blood (table 3, column 6) range from 2.71 to 5.24 mEq./liter, or approximately one-third to three-fifths the muscle lactic acid values. This is evidence that arterial lactic acid, at the time the circulation ceases, has not come into equilibrium with muscle lactic acid, which is formed by anaerobic glycolysis, and diffuses outward into the circulation. These figures furnish a measure of the degree of metabolic acidosis existing in the tissues at the time of death from asphyxia.

At the time of respiratory failure from asphyxia in man, if the CO_2 concentration in the alveolar air is as high as it averaged in the dogs reported here—11 to 13 percent—CO_2 narcosis would be an important factor in respiratory failure. Blood CO_2 content would be so high that the respiratory center would presumably be depressed; vasodilatation and a fall in blood pressure would ensue (19).

It is therefore questionable, whether, under such circumstances, administration of carbogen would have the beneficial effect desired. Rather, it would seem that the additional CO_2 would serve to narcotize the respiratory center to an even greater degree. Until the CO_2 tension of the blood was reduced below the narcotic level, no beneficial effect could be derived from the added CO_2. Before the 'safe' level of CO_2 was attained in the blood, the critical period between cessation of respiration and heart change (about 30 seconds) would possibly have passed; efforts at resuscitation are of no avail a few seconds after the heart has stopped.

SUMMARY

1. Ten healthy anesthetized dogs were asphyxiated by rebreathing limited amounts of room air. A continuous pulmonary ventilation record was made, and electrocardiographic records, arterial blood, and alveolar air samples were taken simultaneously at one- or two-minute intervals during asphyxiation. Analysis of arterial CO_2, oxygen, and lactic acid content, pH, and hematocrit percentages were made. Alveolar samples were analyzed for CO_2 content. Control and death samples of muscle were analyzed for CO_2.

2. The acid-base balance of the blood responded to asphyxia with a respiratory acidosis, and a late metabolic acidosis superimposed. Direct measurements indicated that incoming lactic acid is the cause of CO_2 displacement from the blood during metabolic acidosis.

3. Muscle CO_2 content of dogs is slightly lower at the time of death from asphyxia than it is normally. This decrease in CO_2 content is a measure of the diffusion gradient of lactic acid from tissues to blood and of the degree of metabolic acidosis in the tissues during asphyxia.

4. The initial use of carbogen in resuscitating men subjected to any form of obstructive asphyxia is questioned.

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REFERENCES

1. Wright, S. Applied Physiol. 5th ed. London. Oxford University Press, 1934.
2. Thompson, S. A. and G. L. Birnbaum. Surg. Gyn. Obs. **75**: 79, 1942.
3. Thompson, S. A. and G. L. Birnbaum. Jour. Thor. Surg. **12**: 607, 1943.
4. Grodins, F. S., A. Lein and H. Adler. This Journal **147**: 433, 1946.
5. Fry, F. E. J. Report No. 1, Clin. Invest. Unit 18-3, Project No. A-78 (R. C. A. F.), Apr. 22, 1942.
6. Van Slyke, D. D. and J. M. Neill. Jour. Biol. Chem. **61**: 523, 1924.
7. Barker, S. B. and W. H. Summerson. Jour. Biol. Chem. **138**: 535, 1941.
8. Danielson, I. S. and A. B. Hastings. Jour. Biol. Chem. **130**: 349, 1939.
9. Consolazio, W. V., D. B. Dill and S. M. Horvath. Syllabus of Methods Employed in the Fatigue Laboratory of Harvard Business School and Medical Research Laboratory of Mass. Gen. Hosp. June 1, 1942.
10. Scholander, P. F. Hvalraadets Skrifter Norske Videnskaps-Akademi i Oslo, No. 22, 1940.

11. Scholander, P. F., L. Irving and S. W. Grinnell. Jour. Cell. and Comp. Physiol. **21:** 53, 1943.
12. Grinnell, S. W., L. Irving and P. F. Scholander. Jour. Cell and Comp. Physiol. **19:** 341, 1942.
13. Irving, L., H. C. Foster and J. K. W. Ferguson. Jour. Biol. Chem. **95:** 95, 1932.
14. Shock, N. W. and A. B. Hastings. Jour. Biol. Chem. **104:** 585, 1934.
15. Shock, N. W. and A. B. Hastings. Jour. Biol. Chem. **112:** 256, 1935–36.
16. Henderson, L. J. Blood. New Haven, Yale University Press, 1928.
17. Van Slyke, D. D. and J. Sendroy, Jr. Jour. Biol. Chem. **79:** 781, 1928.
18. Conway, E. J. and P. J. Fearon. Jour. Physiol. **103:** 274, 1944–45.
19. Brown, F. M. U. S. Nav. Med. Bull. **28:** 721, 1930.

OBSERVATIONS OF ARTERIAL OXYGEN CONTENT IN CHILDREN DURING THE INHALATION OF AIR AND 100 PER CENT OXYGEN[1]

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Analyses of arterial blood from 14 normal children have been carried out to determine the percentage oxygen saturation while breathing air and the amount of dissolved oxygen in the blood during the inhalation of 100 per cent oxygen. This investigation was undertaken to provide normal data necessary for the interpretation of studies on children with congenital heart disease. In addition the results of the study appear to indicate that the disparity between observed and theoretical values for arterial oxygen saturation has not yet been adequately explained.

METHODS

Selection of subjects. The children were ambulatory hospital patients without any evidence of cardiac or pulmonary disease. There were 8 boys and 6 girls, whose ages ranged between 7 and 14 years. Studies were carried out on five subjects prior to elective plastic or ocular surgery; on one, three days following an eye muscle operation, and on four others between the seventh and ninth days following appendectomies. One patient had been admitted with diabetes mellitus, one with congenital syphilis, one with possible chorea, and one with possible encephalitis; all were ready for discharge from the hospital when blood was taken for this study.

Technique of arterial puncture. The femoral artery was employed in all cases. The arterial site was determined by palpation, which was facilitated by holding the elected thigh in a position of slight external rotation while the subject was supine. This area, approximately one inch distal to the inguinal ligament, was infiltrated with 2 to 3 cc. of 2 per cent procaine without epinephrine. The puncture was made by using an ordinary lumbar puncture needle provided with a blunted stylet: a #19 gauge needle, 2½ inches in length, was suitable for the older children, whereas a #20 gauge needle, about 1¼ inches in length, was preferable for the younger ones. The needle was attached to a 2 cc., all-glass syringe which had been lubricated previously with paraffin oil and moistened with sufficient 10 per cent potassium oxalate solution to fill the dead space, in the manner described in another communication (1). It was inserted at a 45-degree angle through the anesthetized skin in the line of pulsation; as soon as the needle entered the artery the plunger of the syringe began to move backwards. Approximately 2 cc. of blood were withdrawn and then the syringe was disconnected from the needle and replaced by the stylet. Immediately following the removal of each blood sample the syringe was placed in an ice and water bath until the time of analysis (1). After the final blood sample had been collected and the needle removed from the artery, a dry sponge was held firmly against the site of puncture for several minutes to prevent the formation of a hematoma.

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[2] The data reported in this paper are from a thesis to be presented by this author in partial fulfillment of the requirements for the degree of Doctor of Medicine.

Experimental conditions. All of the selected patients were cooperative and were in a resting state before and during the collection of the blood samples.

1. Subject breathing air. About 10 minutes after the child assumed the recumbent position the arterial puncture was made and the first 2 cc. blood sample was withdrawn.

2. Subject breathing oxygen. While the obturated needle was in the artery, the child breathed 100 per cent oxygen from a snugly fitting, soft rubber mask[3] which was held over the mouth and nose. The gas was delivered from a cylinder at a rate sufficiently rapid to keep the reservoir bag distended throughout the respiratory cycle, thus precluding any leak of air into the system. An inspiratory valve interposed between the reservoir bag and mask and an expiratory valve situated on the dome of the mask operated with negligible resistance and virtually eliminated rebreathing. Oxygen was supplied for a period of three minutes, which was considered sufficient time for the elimination of most of the nitrogen from the lungs (2, 3, 4). It has been shown that after 35 to 40 breaths of normal respiratory volume the alveolar nitrogen is reduced to 2 per cent (3), and that the nitrogen content of the arterial blood is at a constant low level within 2½ minutes (4). The subject continued to breathe oxygen until the second blood sample had been collected.

Analytical procedure. A modified Roughton-Scholander technique of gasometric analysis (1, 5), using 0.04 cc. of blood, was employed in the determination of the oxygen content and capacity of each sample. Duplicate analyses were run simultaneously by two technicians; observed values which checked within 0.2 vol. per cent before reduction to standard conditions were considered satisfactory. The determination of oxygen content was begun as soon as possible on the second sample; there was usually an interval of 15 to 20 minutes between drawing and analyzing the blood. The first sample, collected while the subject was breathing air, was then analyzed for oxygen content. About two hours after the blood had been drawn, a 0.5 cc. aliquot was transferred to a 15 cc. bottle and equilibrated with air for 30 minutes on a mechanical rotator (1), and then the oxygen capacity was immediately determined.[4]

Calculations. Values for oxygen content and capacity were expressed in volumes per 100 cc. of blood under standard conditions of temperature and barometric pressure. From the observed total oxygen capacity the amount of oxygen carried in physical solution (d_{air}) was subtracted (1). The values for total oxygen content and corrected oxygen capacity were increased by 2 per cent to correct for the dilution of the blood sample by the oxalate solution used to fill the dead space of the syringe when the blood was drawn (1). Percentage oxygen saturation was calculated only on the blood drawn while the subject was breathing air, according to the equation,

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[3] Adult Oro-Nasal Meter Mask (Type #720), used without the meter attachment. It is distributed by the Oxygen Equipment Manufacturing Company, 405 East 62nd Street, New York, New York.

[4] In cases 1 and 2 of tables 1 and 2 the blood samples were saturated with oxygen before the equilibration with air, which was accomplished by hand rotation. It is possible that equilibrium between blood and air was not reached in these two cases.

$$O_2 \text{ saturation } (\%) = 100 \times \frac{\text{Total } O_2 \text{ content} - \text{dissolved } O_2}{\text{Corrected } O_2 \text{ capacity}}$$

in which the value for dissolved oxygen in the numerator was taken from a table published elsewhere (1).

Dissolved oxygen (d) is defined as the amount of oxygen in cubic centimeters per 100 ml. of whole blood carried in physical solution in the circulating arterial blood, d_{air} and d_{O_2} representing dissolved oxygen during the inhalation of air and 100 per cent oxygen, respectively. The quantity of dissolved oxygen observed during the inhalation of 100 per cent oxygen, $d_{O_2obs.}$ is equal, by definition, to the total oxygen content minus the corrected oxygen capacity.

EXAMPLE: Case 14 (see table 2)
Total O_2 content = 19.38 vol. per cent
Corrected O_2 capacity = 18.69 vol. per cent
$d_{O_2obs.}$ = 0.69 vol. per cent

The amount of dissolved oxygen that would theoretically be present in a sample of blood equilibrated with oxygen at a tension equal to that in the alveolar air of a person breathing 100 per cent oxygen, is designated $d_{O_2calc.}$ and is calculated as follows:

1. The Bunsen solubility coefficient of oxygen at 38°, α_{38°, is determined for a blood sample of any corrected oxygen capacity according to the following empirical equation of Sendroy, Dillon, and Van Slyke (6):

   $\alpha_{38^\circ} = 0.0209 + 0.000108$ (corrected oxygen capacity in vol. %)
2. The value of α_{38° thus determined is substituted in the formula,

   $$d_{O_2calc.} = \frac{100\,(B - 87)\,\alpha_{38^\circ}}{760}$$

   in which $d_{O_2calc.}$ is dissolved oxygen in volumes per cent, and the expression, B − 87, represents the observed barometric pressure corrected for the tensions of CO_2 (40 mm. Hg) and water vapor (47 mm. Hg) assumed to be present in alveolar air. The small amount of residual nitrogen in the alveoli has been ignored.

Values for $d_{O_2obs.}$ and $d_{O_2calc.}$ were determined for each patient. The difference between them in volumes per cent, $d_{O_2calc.} - d_{O_2obs.}$ appears as Δ in table 2.

The data on blood samples withdrawn during the inhalation of air appear in table 1, and table 2 summarizes the observations made on samples removed while the subjects were breathing oxygen. Values for total oxygen content do not appear in table 2; they are equal to the sums of corresponding values for $d_{O_2obs.}$ and corrected oxygen capacity.

RESULTS

The average arterial oxygen saturation of the 14 normal children while breathing air at rest was 93.1 per cent, with a range of 91.5 per cent to 95.3 per cent (see table 1). During the inhalation of oxygen the total oxygen content usually increased by about 2 vol. per cent, and in all cases the apparent oxygen saturation reached 100 per cent. In *case 4* there was no absolute increase in oxygen content during the inhalation of oxygen; there was, however, a decrease of ap-

TABLE 1. ARTERIAL OXYGEN STUDIES IN NORMAL CHILDREN WHILE BREATHING AIR

CASE	TOTAL O_2 CONTENT	CORRECTED O_2 CAPACITY	O_2 SATURATION
	vol. %	*vol. %*	%
1	19.8_4	20.9_1	$93._8$
2	15.6_6	16.8_5	$91._7$
3	15.9_6	17.1_8	$91._7$
4	17.4_7	18.3_8	$93._8$
5	14.5_4	15.4_4	$92._7$
6	15.0_6	15.8_5	$93._6$
7	16.0_7	16.8_8	$93._8$
8	14.4_8	15.4_3	$92._5$
9	18.0_2	19.2_4	$92._6$
10	16.4_7	17.2_9	$93._9$
11	15.6_5	16.6_2	$92._8$
12	15.8_0	16.3_2	$95._3$
13	14.6_0	15.7_4	$91._5$
14	17.5_7	18.4_9	$93._8$
AVERAGE	16.23	17.19	93.1

TABLE 2. DISSOLVED OXYGEN IN THE BLOOD OF NORMAL CHILDREN DURING THE INHALATION OF 100 PER CENT OXYGEN

CASE	CORRECTED O_2 CAPACITY	d_{O_2}		
		d_{O_2} obs.	d_{O_2} calc.	Δ
	vol. %	*vol. %*	*vol. %*	*vol. %*
1	20.0_1	1.0_3	2.0_5	1.0_2
2	16.5_1	0.9_5	1.9_7	1.0_2
3	16.9_1	1.0_8	2.0_1	0.9_3
4	16.3_7	1.0_9	2.0_0	0.9_1
5[1]	(12.7_6)	(0.9_9)	(1.9_7)	(0.9_8)
6	16.0_8	1.1_1	1.9_9	0.8_8
7	16.9_1	1.0_0	2.0_1	1.0_1
8	15.1_0	1.6_6	1.9_8	0.3_2
9	19.3_6	0.8_9	2.0_2	1.1_3
10	17.0_2	1.4_6	2.0_2	0.5_6
11	16.7_0	1.0_6	2.0_2	1.0_2
12	16.2_1	1.3_4	2.0_0	0.6_6
13	15.6_4	0.9_2	1.9_8	1.0_6
14	18.6_9	0.6_9	2.0_1	1.3_2
AVERAGE......	17.04	1.09	2.00	0.91
STANDARD DEVIATION............		±0.26		±0.26

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[1] The results of analyses in *Case 5* were not included in the averages because of possible error due to blood blotting.

proximately 2 vol. per cent in the oxygen capacity of the second blood sample, so that the oxygen saturation was complete.

The average value for the observed amount of dissolved oxygen in the blood during the inhalation of pure oxygen was 1.09 vol. per cent (see table 2). The result of the determination of $d_{O_2obs.}$ in *case 8* seems disproportionately high, though the analyses appeared satisfactory; if this case were omitted the average $d_{O_2obs.}$ would equal 1.05 vol. per cent.

The theoretical value for the quantity of dissolved oxygen was in each case higher than actually observed. This discrepancy, indicated by Δ, averaged 0.91 vol. per cent.

DISCUSSION

The average figure for oxygen saturation, 93.1 per cent, is 0.8 to 2.9 per cent lower than other reported averages (7–12). One of the earlier investigations (7), in which a colorimetric method was employed, was carried out on 28 normal children; an average value of 94.7 per cent was reported with a range of 91 to 98 per cent. In the other studies, carried out on adults, standard gasometric procedures were used, but it is not always clear from the reports that corrections were made for dissolved oxygen. In the present study an average figure of 0.22 vol. per cent for dissolved oxygen was subtracted from the total oxygen content; had this correction not been made our average saturation would have been calculated as 94.4 per cent. All of the children were ambulatory, and many were given deep breathing exercises before the arterial sampling procedure was begun. Any reduction in percentage saturation brought about by deficient pulmonary ventilation and atelectasis induced by bed rest (3, 9) was thus avoided.

Our average arterial saturation of 93.1 per cent is 5.5 per cent lower than the average value of 98.6 per cent determined spectrophotometrically by Drabkin and Schmidt (13) on freshly drawn, oxalated, hemolyzed, human arterial blood taken while the subjects were breathing air. Our average difference of 0.91 vol. per cent between $d_{O_2obs.}$ and $d_{O_2calc.}$ indicates that during the inhalation of oxygen, the observed oxygen content was likewise lower than its predicted value, by an average 5.3 per cent. The discrepancy between empirical and theoretical values for arterial oxygen saturation has been attributed by Roughton, Darling, and Root (11) to factors inherent in the gasometric determination of oxygen capacity. The two factors of importance are differential drainage of cells and plasma from the saturating vessel and spontaneous reversion of a fraction of the hemoglobin from a form which is inactive *in vivo* but becomes capable of combining with oxygen after being withdrawn from the body. In the present study drainage factors were held to a minimum in the determination of oxygen capacity, leaving the reversion of inactive hemoglobin as a plausible explanation for the discrepancies in our figures. However, actual measurements in other laboratories of the carbon monoxide-combining power of blood immediately after its removal from the body and again an hour or two later indicate an average spontaneous increase in carbon monoxide capacity of only 0.4 (14) to 1.1 (11) per cent.

The answer to the problem of discrepancies between observed and theoretical contents of oxygen in the circulating arterial blood may be resolved by a study in which determinations of arterial oxygen saturation by both gasometric and spectrophotometric methods are carried out on the same specimens of blood. The studies of arterial oxygen tension carried out by Comroe and Dripps on patients breathing air (15) and by Berggren on individuals breathing 100 per cent oxygen (3) leave little doubt that arterial blood is normally in equilibrium with alveolar air. The hypothesis of Roughton, Darling, and Root (11), that deviation of gasometric results from the theoretical must be attributed to changes in stored blood, is thus supported by good indirect evidence. However, their explanations for these changes reconcile the entire discrepancy neither in the present study nor in those investigations in which higher average values for arterial oxygen saturation are reported. Apparently the manipulations attendant to saturating blood with oxygen or carbon monoxide bring about an increase in the gas-combining capacity of the blood.

It is apparent from table 2 that over the small range of oxygen capacities observed in this study—viz., 15–20 vol. per cent—there is no demonstrable correlation between oxygen capacity and $d_{O_2 obs}$. Theoretical variations in d_{O_2} with oxygen capacity would, however, be too small to be detected by the analytical techniques we have employed, as is evident from the values for $d_{O_2 calc}$. in table 2.

The results of these experiments find practical application in the diagnosis of congenital heart disease. Knowledge of the average amount of oxygen carried in physical solution in the pulmonary venous blood during the inhalation of oxygen has made it possible to estimate quantitatively the extent of impairment of pulmonary diffusion of oxygen as well as the shunting of venous blood into the systemic circulation, in patients with arterial anoxemia (16).

It must of course be borne in mind that the values here reported are defined in terms of the analytical techniques employed.

SUMMARY

A technique of femoral artery puncture has been described. Arterial blood samples were obtained from 14 normal children and the results of oxygen analyses by a modified Roughton-Scholander technique have been reported.

The average arterial oxygen saturation under conditions of rest and the breathing of room air was found to be 93.1 per cent, with a range of 91.5 to 95.3 per cent.

The average amount of oxygen in physical solution in the arterial blood during the inhalation of 100 per cent oxygen was found to be 1.09 vol. per cent, or 55 per cent of the amount theoretically expected. The difference between the theoretical and observed values is of the same order of magnitude as the discrepancy between the theoretical and observed values for arterial oxygen saturation during the inhalation of air. The possible mechanisms that produce these discrepancies have been discussed.

We wish to express our deep appreciation and sincere thanks to Miss Dorothy Nixon for her technical assistance and cooperation in this project.

REFERENCES

1. Ordway, N. K. To be published.
2. Darling, R. C., A. Cournand and D. W. Richards, Jr. J. Clin. Investigation **23:** 55, 1944.
3. Berggren, S. M. Acta Physiol. Scand. **4:** Supp. 11, 1942.
4. Ferris, E. B., Jr., and M. A. Blankenhorn. Nat. Res. Council, Div. of Med. Sci., Committee on Aviation Medicine, Report No. 4, May 29, 1942.
5. Roughton, F. J. W. and P. F. Scholander. J. Biol. Chem. **148:** 541, 1943.
6. Sendroy, J. Jr., R. T. Dillon and D. D. Van Slyke. J. Biol. Chem. **105:** 597, 1934.
7. Brinkman, R. and J. H. P. Jonxis. Acta Med. Scand. **94:** 453, 1938.
8. Gibbs, E. L., W. G. Lennox, L. F. Nims, and F. A. Gibbs. J. Biol. Chem. **144:** 325, 1942.
9. Cullen, S. C. and E. V. Cook. This Journal **137:** 238, 1942.
10. Keys, A. and A. M. Snell. J. Clin. Investigation. **17:** 59, 1938.
11. Roughton, F. J. W., R. C. Darling and W. S. Root. This Journal **142:** 708, 1944.
12. Cournand, A., R. L. Riley, E. S. Breed, E. deF. Baldwin and D. W. Richards, Jr. J. Clin. Investigation **24:** 106, 1945.
13. Drabkin, D. L. and C. F. Schmidt. J. Biol. Chem. **157:** 69, 1945.
14. Van Slyke, D. D., A. Hiller, J. R. Weisiger and W. O. Cruz. J. Biol. Chem. **166:** 121, 1946.
15. Comroe, J. H., Jr. and R. D. Dripps, Jr. This Journal **142:** 700, 1944.
16. Ordway, N. K. To be published.

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